EXPLORING THE AETIOLOGY OF ADHD: RATER EFFECTS, CO-OCCURRING TRAITS AND POLYGENIC SCORES

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ABSTRACT

The precise aetiology of attention-deficit/hyperactivity disorder (ADHD) and its association with co-occurring traits remains unclear. Accordingly, the overarching aim of this thesis was to address several ambiguities surrounding the causes and correlates of ADHD. The first of these ambiguities concerns rater effects in twin studies. This was addressed by examining parent, teacher and child self-ratings of ADHD symptoms obtained concurrently using population-based twin data. Results revealed significantly lower heritability for self-ratings than for parent or teacher ratings of ADHD symptoms, but also identified a common genetic basis for the different informant ratings of ADHD-related behaviours. The second of these ambiguities concerns the association between ADHD and Cloninger's dimensions of temperament, examined in a population-based sample of adult twins. Results revealed heterogeneity in the phenotypic and aetiological associations of hyperactivity-impulsivity and inattention with the different dimensions of temperament. The third of these ambiguities concerns the relationship between ADHD and emotional lability. This was initially addressed in a twin study of children and adolescents. Results revealed significant phenotypic associations and a common genetic basis for symptoms of hyperactivity-impulsivity, inattention and emotional lability. A second study examined the association of the same symptom dimensions with measures of cognitive performance in child twin pairs. Phenotypic and genetic analyses indicated no direct association between cognitive performance and emotional lability after controlling for the symptoms of ADHD. The fourth of these ambiguities concerns the disparity between quantitative and molecular genetic studies of ADHD. This was addressed by testing the polygenic theory of ADHD. A polygenic profile score was generated using genome-wide association results derived from a large discovery sample of ADHD cases and controls. The profile score was significantly associated with ADHD affection status and with ADHD symptom scores in independent samples. The implications of these findings and future directions for research are discussed.

STATEMENT OF AUTHORSHIP

The research described in this thesis was undertaken using secondary data from a number of studies. I am grateful to the primary investigators for providing me with access to these datasets and to the participating families.

The results reported in Chapter 3 are based on data from the Twins Early Development Study (TEDS), led by Professor Robert Plomin and supported by a programme grant (G0500079) from the UK Medical Research Council (MRC) and by a grant (HD044454) from the US National Institutes of Health (NIH).

The results reported in Chapter 4 are based on data from the Swedish Twin Study of Child and Adolescent Development (TCHAD), led by Professor Paul Lichtenstein and supported by funding from the Swedish Council for Working Life and Social Research (2004-0383) and the Swedish Research Council (2004-1415).

The results reported in Chapter 5 are based on data from the Cardiff Study of All Wales and North West of England Twins (CASTANET), led by Professor Anita Thapar and supported by funding from the UK MRC (G9806217).

The results reported in Chapter 6 are based on data from the Study of Activity and Impulsivity Levels in Children (SAIL), led by Dr Jonna Kuntsi and supported by funding from the Wellcome Trust (GR070345MF).

The results reported in Chapter 7 use data from TEDS and SAIL, in addition to data from the Psychiatric Genomics Consortium (PGC, formerly Psychiatric GWAS consortium) ADHD subgroup. The PGC was established by Professor Patrick F. Sullivan and supported by funding from the US National Institutes of Health (1U01MH085520-01). The PGC ADHD subgroup is led by Professor Stephen V. Faraone and includes principal investigators from nine international studies.

My PhD studentship was supported by a studentship grant (ES/H012354/1) from the UK Economic and Social Research Council (ESRC).

I was responsible for formulating hypotheses and conducting statistical analyses across the datasets used. The work reported throughout this thesis is original research and represents my own work, with the following exceptions:

1. The review of the literature on temperament and ADHD in chapter 1 was assisted by the comprehensive literature search completed by Ms Simrit Nijjar, an undergraduate medical student whom I supervised to complete a systematic review of the genetic associations between ADHD and Cloninger's temperament dimensions. I supervised Ms Nijjar's work on this topic, devised the research question and search strategy for the review and have taken a lead in writing up a review paper for publication. All work presented in chapter 1 is therefore my own.

2. The univariate analyses of parent, teacher and self-ratings of ADHD reported in chapter 3 were originally completed as part of my Masters of Science (MSc) degree in Social, Genetic and Developmental Psychiatry, between September 2009 and June 2010. For my PhD the univariate analyses were extended to examine same versus different teachers and all multivariate analyses were conducted.

3. The examination of emotional lability reported in chapter 4 arose as a result of factor analysis completed in the same dataset by my collaborator, Dr Wai Chen, for his PhD submitted in 2011. I am solely responsible for all phenotypic and genetic analyses reported in this thesis.

4. The polygenic analyses reported in chapter 7 used genome-wide genotype data from TEDS and the PGC. These data had already been pre-processed to enable genome-wide analyses, with credit to Mr Maciek Trzaskowski from the TEDS team and Dr Benjamin Neale from the PGC for their work in preparing the data. I describe the data preparation in the methods chapter of this thesis but take no credit for this work. However I am responsible for the polygenic profile scoring and analyses reported in chapter 7.

As a PhD student I was involved in a number of additional studies not included in this thesis, but which included the collection of primary data. From 2010 to 2011 I was responsible for co-ordination of the Adult ADHD Genetics study, which aims to identify common and rare genetic variants associated with adult ADHD. I coordinated the collection of biological samples and phenotypic data from patients referred to the National Adult ADHD Clinic at South London and Maudsley (SLAM) Hospital, conducted data entry and analysis, and supervised placement students working on this project. I also regularly attended and contributed to weekly clinic meetings and quarterly research meetings of SLAM's National Adult ADHD Service and disseminated research findings to clinicians via the King's Health Partners' Behavioural and Developmental Disorders (BDD) Clinical Academic Group (CAG).

In 2012 I conducted a qualitative research project, entitled *"The Advantages of ADHD".* This study sought to determine whether some adults with ADHD perceive strengths as well as difficulties associated with their ADHD symptoms. The primary investigator for this research was Professor Philip Asherson and the study formed part of a wider qualitative investigation of adult ADHD patient experiences in service provision and clinical management. The study was undertaken in collaboration researchers at University College London and with support from Shire Pharmaceuticals. My role in this project involved generating research questions specific to positive psychology and ADHD, creating and piloting a qualitative interview schedule, recruiting participants via the National Adult ADHD Service at SLaM, and conducting semi-structured research interviews with participants. I have now begun to analyse the data and hope to continue research into positive psychology and ADHD in future.

PUBLICATIONS

Sections of chapter 1 are adapted from the following publication:

Merwood, A., Asherson, P. (2011). Attention deficit hyperactivity disorder: A lifespan genetic perspective. *Advances in Mental Health and Intellectual Disabilities, 5,* 33-46.

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Merwood, A., Larsson, H., Rijsdijk, F., Chen, W., Asherson, P. (in preparation). A common aetiology for ADHD, aggression and anxious-depressed symptoms in adulthood twins. *Target Journal: American Journal of Psychiatry.*

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Nijjar, S., **Merwood, A.,** Reid, P., Skirrow, C., Asherson, P. (in preparation). The Cognitive Control Questionnaire: A new measure of mind-wandering in adult ADHD. *Target Journal: ADHD Attention Deficit and Hyperactivity Disorders.*

Reid, P., **Merwood, A.,** Skirrow, C., Asherson, P. (in preparation). A meta-cognitive model of depression in ADHD. *Target Journal: ADHD Attention Deficit and Hyperactivity Disorders.*

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Although not a collaborator on any single project I owe a debt of thanks to Mr Stuart Newman, who went beyond the call of duty when teaching me to use the cluster computer at the SGPD; his ability to solve any number of problems with a few lines of Bash scripting left me astounded. I would also like to say thank you to Dr Helena Zavos for answering my many, many questions over the years and for all the little conversations that came with the answers.

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throughout this time, Steph, Helena, Corina, Ruth, Sania, Luciana, Glenn, Magda and Helen, who made coming into work such a joy. Rob, although *technically* not an office-mate, would frequently wander in and deserves a share of the credit here, too.

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Saving the best to last, I would like to thank Vanessa for her love, friendship and support. This thesis is dedicated to her.

1. BACKGROUND LITERATURE

1.1 OVERVIEW

Attention-deficit/hyperactivity disorder (ADHD) is a common, complex neurodevelopmental disorder, characterised by difficulties in sustaining attention (inattention), restless, overactive behaviours (hyperactivity), and poor impulse control (impulsivity). This is, however, a narrow conceptualisation of a highly prevalent disorder (see section 1.3.2) that is associated with a wide range of impairments and comorbidities throughout the lifespan (see section 1.3.5) and with symptoms that also present at a trait-like level throughout the general population (see section 1.3.1). The term ADHD can therefore be seen as referring to a broad, complex and highly heterogeneous phenotype comprising symptoms of hyperactivity-impulsivity and inattention at its core.

Because of the complexity of ADHD, much remains to be understood about the causes and correlates of the disorder. There is a need to understand inconsistencies in genetic research. For example, the heritability of ADHD is widely reported as ranging from 70-80%, yet heritability estimates range as low as zero when twin studies utilise self-reports of symptoms (see section 1.4). Similarly, despite the high heritability, genome-wide association studies have failed to identify markers significantly associated with the disorder at the genome-wide level (see section 1.5). Addressing these questions will improve understanding of how and why ADHD occurs. There is also a need to understand how ADHD symptoms relate to other traits. This includes dimensions of temperament, which if phenotypically and aetiologically associated, might be used to characterise more homogeneous subtypes of ADHD in future (see section 1.7). This also includes understanding the aetiological association with emotional lability, which has been increasingly linked to ADHD in clinical studies (see section 1.8).

The literature review in this chapter aims to set the scene for addressing these questions, providing a comprehensive overview of key research findings to

date. The specific aims of this thesis are then outlined along with details of the research questions to be addressed.

1.2 PSYCHOPATHOLOGY

1.2.1 Past, present and future

ADHD has received more than its fair share of controversy. Until relatively recently it was condemned by some parts of the media as a *"disorder of the '90s"* (Anastopoulos and Shelton, 2001), leading to criticism and confusion, even hostility, within the public domain (Mayes et al., 2008). Misgivings remain over the extent of stimulant medication use (Mayes et al., 2008) and the validity of adult ADHD (Moncrieff and Timimi, 2010), but in general these have subsided such that the lay view is increasingly in line with the overwhelming scientific consensus that ADHD is, and always has been, a valid psychiatric disorder necessitating clinical treatment and management at all stages of life (Asherson et al., 2010, Barkley, 2002, Kooij et al., 2010, NICE, 2008).

This shift in opinion is in line with historic descriptions of the core symptoms of ADHD, defined across disciplines of psychology, psychiatry, pediatrics and neurology as far back as 1798 (Lange et al., 2010). A gradual, empirical refinement of these descriptions led to the development of formal diagnostic criteria, as set out in the Diagnostic and Statistical Manual of Mental Disorders fourth edition and its text revision (DSM-IV, DSM-IV-TR; American Psychiatric Association, 1994, 2000) and described in the International Classification of Diseases – Tenth Edition (ICD-10; World Health Organisation, 1996). The DSM criteria have now been revised in the fifth edition, published in May 2013 (DSM-5, American Psychiatric Association, 2013). Given this vantage it seems relevant to consider the past, present and future when describing the psychopathology of ADHD.

Historic conceptualisations (*the past*) of what is now known as ADHD have been consistently reviewed (Barkley, 2010, Lange et al., 2010, Taylor, 2011, Warnke and Riederer, 2013) and are summarised briefly here. The first medical account is regarded as a description of attention problems by Alexander

Crichton, published in 1798. This work described a state of poor attention and impaired learning, present from birth but diminishing over time, that is strikingly similar to the modern concept of ADHD. Similar observations were recorded throughout the 1800s by medical doctors including Haslam (1809), Rush (1812), Esquirol (1845) and Clouston (1899), culminating in George Still's account of *"moral control"* published in *the Lancet* in 1902. These varying descriptions included at their core the symptoms of inattention, hyperactivity, impulsivity and dysregulation of emotions, which were considered severely impairing to the individual. William James, one of the fathers of clinical psychology, also described the need for psychology to overcome *"wandering attention"* around this time (James, 1890), while Heinrich Hoffman's *Fidgety Phil* provides an entertaining, if not entirely accurate, literary description of the hyperactive behaviour associated with ADHD (Hoffmann, 1845, Taylor, 2011).

Research and recognition of ADHD continued throughout the 20th century (Barkley, 2010, Lange et al., 2010, Taylor, 2011, Warnke and Riederer, 2013). Notable milestones included the introduction of the concept of *minimal brain damage* in 1908 and its replacement with the concept of *minimal brain dysfunction* in the 1960s-70s, the latter of which increasingly emphasised the role of attentional processes. This period also saw the formal classification of inattentive, hyperactive and impulsive behaviours, termed *hyperkinetic reaction of childhood* in DSM-II (1968), *attention-deficit disorder* in DSM-III (1980) and, finally, *attention deficit hyperactivity disorder* in DSM-III-TR (1987). The genesis of related terminology in ICD-8 (*hyperkinetic reaction of childhood*) and ICD-9 (*hyperkinetic syndrome*) also occurred throughout this time.

The most recent definition of ADHD (*the present*) was based on the criteria set out in the fourth edition of DSM and its subsequent text revision (DSM-IV, DSM-IV-TR; American Psychiatric Association, 1994, 2000). This definition outlines 18 core symptoms of hyperactivity-impulsivity and inattention. The same symptoms are also detailed in the criteria for *hyperkinetic disorder* in ICD-10 (World Health Organisation, 1992). These criteria have informed, and been informed by, almost 20 years of clinical practice and research, including the original research conducted for this thesis (chapters 3-8). The full extent of the current diagnostic criteria are considered in section 1.2.2.

Now published, DSM-5 (American Psychiatric Association, 2013) can be considered *the future* of ADHD, as it has yet to fully influence clinical practice and major research. Yet DSM-5 makes very few changes when compared to DSM-IV. The same 18 items are retained, albeit with developmentally-appropriate symptom descriptions for adults, a reduction in the number of symptoms required in adulthood, changes to the age of onset criteria and allowance for the co-occurrence of autism spectrum disorders. These criteria are also described in section 1.2.2.

1.2.2 Diagnostic criteria and clinical guidelines

There are no gold standard biogenic tests for the detection of ADHD. Therefore the most widely used diagnostic criteria are behavioural descriptions such as those published in DSM-IV and its subsequent revisions (DSM-IV-TR, DSM-5) and in ICD-10. Both DSM-IV/5 and ICD-10 identify 18 behavioural symptoms corresponding to two core dimensions: hyperactivity-impulsivity (9 items) and inattention (9 items). Individual items are presented in Table 1.1. The validity of the two dimensions is supported in factor-analytic research, including the recently defined bi-factor model of ADHD. The bi-factor model identifies two separate factors for the hyperactive-impulsive and inattentive dimensions, in addition to a general factor that accounts for the range of symptoms across both dimensions (Martel et al., 2011, Martel et al., 2010c, Toplak et al., 2009). This structure appears invariant across informant, age and cultural setting (Toplak et al., 2012), indicating a consistent relationship between the two domains.

Based on DSM-IV criteria (including DSM-IV-TR), a diagnosis of ADHD is made when an individual endorses six or more symptoms in either the hyperactive-impulsive or inattentive domain. Six or more symptoms of inattention correspond to a diagnosis of predominantly-inattentive ADHD, six or more hyperactive-impulsive symptoms correspond to a diagnosis of predominantly-hyperactive/impulsive ADHD and six or more symptoms in both domains correspond to a diagnosis of combined-type ADHD. DSM-IV also outlines additional criteria that must be met in order for an ADHD diagnosis to be made:

The onset of several symptoms and some impairment must occur prior to seven years of age; the symptoms must be pervasive across settings (i.e. presentations at home and school); the symptoms must cause significant functional impairments in everyday life; and the symptoms should not occur exclusively during the course of a pervasive developmental disorder, schizophrenia or another psychotic disorder, or be better accounted for by another disorder. In both DSM-IV and DSM-5, symptoms of emotional lability (i.e. mood volatility, irritability) are outlined as associated features of ADHD, although it is increasingly argued that such symptoms may reflect a core component of the disorder (see section 1.8).

The ADHD criteria were only slightly changed in DSM-5. Changes include removal of the exclusion criteria preventing individuals with autism from receiving a diagnosis (consistent with patterns of comorbidity reported in section 1.3.5) and the removal of diagnostic subtypes. For example, predominantly-inattentive cases of ADHD are now *inattentive presentations*, based on the premise that the precise pattern of ADHD symptoms fluctuates over time (see section 1.3.4). DSM-5 additionally sets out amended diagnostic criteria for adults. The lack of adult diagnostic criteria was first recognised by Paul Wender in 1995 and subsequently addressed in clinical guidelines for adult ADHD (Asherson, 2005, Haavik et al., 2010, Kooij et al., 2010, NICE, 2008, Wender, 1995). The relevant amendments in DSM-5 include age-appropriate changes to the wording of individual items, revised thresholds for the number of symptoms required (5 rather than 6, in individuals aged 17 or older) and relaxed age of onset criteria (12 rather than 7 years of age), and allowing for impairments to develop after the age of onset of symptoms by age 12. These changes should facilitate the diagnosis of adult ADHD and are in line with recently published clinical guidance (Haavik et al., 2010, Kooij et al., 2010, NICE, 2008)

In ICD-10, the definition of hyperkinetic disorder essentially includes the same 18 items listed for ADHD in DSM-IV (table 1.1), but with some differences in item wording. However, there are also distinctions between the two diagnostic systems. First, ICD-10 identifies five hyperactive and four impulsive items (*"talks excessively"* is considered impulsive). Second, ICD-10 requires that at least six inattentive items, three hyperactive and one impulsive item be endorsed in

order for hyperkinetic disorder to be diagnosed. This means that hyperkinetic disorder most closely resembles combined-type ADHD. Accordingly, research shows that ICD-10 criteria lead to diagnosis of fewer individuals with hyperkinetic disorder than would otherwise be diagnosed with ADHD based on DSM-IV, and that those identified have more severe levels of symptoms and impairments (Lahey et al., 2006, Lee et al., 2008, Dopfner et al., 2008). Other criteria regarding age of onset and comorbidities are more or less the same.

Table 1.1 The 18 diagnostic items for ADHD

	Inattention
1	Often fails to give close attention to details or makes careless mistakes in schoolwork, work, or other activities
2	Often has difficulty sustaining attention in tasks or play activities
3	Often does not seem to listen when spoken to directly
4	Often does not follow through on instructions and fails to finish schoolwork, chores, or duties in the workplace (not due to oppositional behaviour or failure of comprehension)
5	Often has difficulty organizing tasks and activities
6	Often avoids, dislikes, or is reluctant to engage in tasks that require sustained mental effort (such as schoolwork or homework)
7	Often loses things necessary for tasks or activities at school or at home (e.g. toys, pencils, books, assignments)
8	Is often easily distracted by extraneous stimuli
9	Is often forgetful in daily activities
	Hyperactivity
10	Often fidgets with hands or feet or squirms in seat
11	Often leaves seat in classroom or in other situations in which remaining seated is expected
12	Often runs about or climbs excessively in situations in which it is inappropriate (in adolescents or adults, may be limited to subjective feelings of restlessness)
13	Often has difficulty playing or engaging in leisure activities quietly
14	Often talks excessively
15	Is often ‘on the go’ or often acts as if ‘driven by a motor’
	Impulsivity
16	Often has difficulty awaiting turn in games or group situations
17	Often blurts out answers to questions before they have been completed
18	Often interrupts or intrudes on others, e.g. butts into other children's games

Note: Items replicated from DSM-IV-TR (American Psychiatric Association, 2000); hyperkinetic disorder items in ICD-10 are the essentially same, but with subtle differences in some wordings and with item 14 listed as a hyperactive symptom.

In practice, neither diagnostic manual is used in isolation and most health services provide additional guidelines on the diagnosis of ADHD. In the UK, these guidelines are published by the National Institute of Health and Clinical Excellence (NICE, 2008). NICE guidelines recommend that ADHD be diagnosed based on the severity of symptoms and the degree to which they cause impairment. Determining severity is described as a matter of clinical judgement, which should be established based on a semi-structured clinical interview with the individual and/or their family members, depending on developmental stage. The advantage of clinical interviews over questionnaires is that examples of specific symptoms and the impairments caused can be sought; however informant or self-report questionnaires are useful as an initial screening tool and for determining the severity of symptoms.

NICE guidelines additionally make recommendations for the treatment and management of ADHD, advocating pharmacological and/or non-pharmacological interventions depending on developmental stage. These recommendations are based on empirical research. The strongest evidence of treatment effects is found for medication. Meta-analyses indicate moderate-to-good effect sizes for pharmacological treatments of ADHD using stimulant and non-stimulant medications throughout the lifespan (Faraone et al., 2006b, Faraone and Buitelaar, 2010, Faraone et al., 2004, Meszaros et al., 2009).

In contrast, the efficacy of non-pharmacological interventions is less clear-cut. A recent meta-analysis of child and adolescent treatment studies found significant improvements in ADHD symptoms in response to dietary and psychological interventions, including diet restrictions, fatty acid supplementation, neurofeedback, cognitive training and behavioural interventions (Sonuga-Barke et al., 2013). However, when using blinded ratings of ADHD symptoms as the outcome measure only free fatty acid food supplementation and artificial food colour exclusion led to a significant reduction in symptoms. A review of non-pharmacological treatments suggests a potentially beneficial role for CBT in the treatment of adolescent and adult ADHD, although controlled trials are required to fully endorse this approach (Young and Myanthi Amarasinghe, 2010). Studies that have combined pharmacological and non-pharmacological interventions tend to show a preferential effect of multimodal treatments over

non-pharmacological interventions alone (Young and Myanthi Amarasinghe, 2010). Research has only recently begun to explore the merits of mindfulness-based therapies for ADHD, however emerging evidence suggests a potential role in reducing core symptoms and residual impairments across the lifespan (van de Weijer-Bergsma et al., 2012, van der Oord et al., 2012, Zylowska et al., 2008).

1.3 EPIDEMIOLOGY

1.3.1 A clinical disorder and a continuous trait

The criteria described in section 1.2.2 are used to make a clinical diagnosis of ADHD, where an individual is categorised as either affected or unaffected. These criteria are undoubtedly important in identifying individuals with severe ADHD symptoms who are impaired and who will likely benefit from treatment. However, a categorical classification can be seen as somewhat arbitrary, since research has consistently demonstrated that the ADHD symptoms are also trait-like (Frazier et al., 2007, Haslam et al., 2006, Lubke et al., 2009). These studies find no qualitative differences between those with clinical levels of ADHD symptoms and the remainder of the population, and that instead indicate quantitative distinctions, whereby individuals with ADHD present with more severe symptoms and associated impairments in a linear fashion.

The continuous distribution of ADHD symptoms is further supported by research into the Strengths and Weaknesses of ADHD and Normal Behavior Rating Scales (SWAN), designed to measure ADHD symptoms across the continuum (Swanson et al., 2006). The SWAN is different to standard ADHD rating scales since it measures strengths as well as deficits in attention, activity and impulse control, finding a near-normal distribution of symptoms throughout the population (Arnett et al., 2013, Hay et al., 2007, Polderman et al., 2007, Young et al., 2009a). A key strength of the continuous approach to understanding ADHD is that it allows large, population-based samples, unselected for clinical extremes, to be used in epidemiological and aetiological research.

1.3.2 Prevalence

The prevalence of ADHD has been robustly estimated. A recent meta-regression analysis of 171,756 children and adolescents from 102 studies estimated worldwide ADHD prevalence of 5.29%. In these analyses there was significant heterogeneity in the prevalence of ADHD, based on the use of community versus school samples, parent versus teacher ratings of symptoms, the inclusion of impairment criterion, and the use of DSM versus ICD diagnostic criteria. Geographical region had only a modest effect on prevalence, with lower estimates in North Africa and the Middle East when compared the North America. This suggests that ADHD is largely invariant across culture but that methodological differences influence overall prevalence, highlighting the importance of establishing pervasiveness of symptoms and impairment when diagnosing ADHD.

Meta-regression analysis of data from six samples estimates lower prevalence of 2.5% for adult ADHD (Simon et al., 2009). This study also found evidence of significantly lower prevalence with increasing age. However, a prevalence estimate of 6.2% was recently obtained in a study of middle-aged adults (Das et al., 2012), while individual studies excluded from analyses by Simon et al. (2009) also estimated higher prevalence rates for adult ADHD, of 4.4-5.2% (Fayyad et al., 2007, Kessler et al., 2006). As with the childhood data, methodological variation likely accounts for much of the heterogeneity across adult studies. Further research is therefore required to generate a robust estimate of the prevalence of adult ADHD.

1.3.3 Sex effects

Sex differences have been reported in most clinical studies of child and adolescent ADHD, with higher prevalence rates among boys than girls (Gaub and Carlson, 1997, Gershon, 2002, Novik et al., 2006, Rucklidge, 2008) and evidence of higher prevalence of both the hyperactive-impulsive and inattentive subtypes among boys (Ford et al., 2003). Patterns of psychiatric comorbidity have also been reported to differ as a function of sex, with greater levels of externalising problems among boys and greater internalising problems among

girls (Biederman et al., 2002, Ford et al., 2003, Gershon, 2002, Rucklidge, 2008). However, some recent studies using population-based samples have failed to identify sex differences in the prevalence of child and adolescent ADHD, arguing that this difference could be the result of a referral bias in clinical populations (Alloway et al., 2010, Biederman et al., 2005b). This conclusion is not supported by the results from population-based twin samples, which have consistently identified higher mean symptom scores for hyperactive-impulsive, inattentive and total ADHD symptoms among boys (Goodman and Stevenson, 1989a, Greven et al., 2011c, Larsson et al., 2006).

Studies of adult ADHD indicate similar prevalence rates, diagnostic subtypes and patterns of comorbidity among men and women (Biederman et al., 2004, Friedrichs et al., 2012), suggesting that the preponderance of ADHD among males does not persist across the lifespan. However, another study identified a shift in the pattern of sex differences whereby higher ADHD prevalence rates, levels of impairment and comorbidity were found for women as opposed to men (Robison et al., 2008). The extent to which sex differences in ADHD truly subside over the course of development therefore remains unclear. It should also be noted that the extent to which there are sex differences in the aetiology of ADHD is a separate research question (see section 1.4.4).

1.3.4 Age and developmental trajectories

ADHD symptom presentation is not entirely stable over time. A meta-analysis of longitudinal studies found that only 15% of adults retained a diagnosis of ADHD from childhood; however 65% of adults retained either full or sub-syndromal levels of symptoms and associated functional impairments (Faraone et al., 2006a). This suggests that although there is a clear, age-related decline in ADHD symptoms from childhood to adulthood, the criteria used to assess and diagnose ADHD influences rates of persistence and remission. Predictors of ADHD persistence include levels of psychiatric comorbidity, impairment and maternal psychopathology in boys (Biederman et al., 2011, Biederman et al., 2010). The same predictors, in addition to performance in school, were also associated with ADHD persistence in girls (Biederman et al., 2012b). Some studies indicated a possible change in ADHD symptom presentation over time,

with greater rates of inattention in adults; however this was not consistently the case for males (Biederman et al., 2010, Biederman et al., 2012b). These results are therefore only partially in line with prior research indicating a greater role of inattention in adult ADHD (Millstein et al., 1998). In contrast, a recent study of developmental trajectories, using population-based data, identified two trajectories for hyperactivity-impulsivity (stable-low and high-decreasing) and two for inattention (stable-low and low-increasing), indicating an increase in inattentive symptoms from childhood through to late adolescence (Larsson et al., 2011). Another study similarly classified low, increasing and decreasing trajectories of ADHD across childhood and adolescence (Robbers et al., 2011). The extent to which the stability and change in ADHD is due to genetic/environmental factors has been examined via twin research (see section 1.4.5).

1.3.5 Comorbidity

ADHD is linked to a number of psychiatric comorbidities throughout the lifespan. In childhood, common comorbidities include conduct problems (14-15%), oppositional-defiant disorder (45-55%), major depression (42-50%), bipolar disorder (9-13%), and anxiety disorders (29-33% of children have more than two) (Busch et al., 2002). Somewhat lower rates are reported in adulthood, with comorbidities of major depression (18.6%), dysthymia (12.8%) bipolar disorder (19.4%), any anxiety disorder (47.1%), any substance use (15.2%) and intermittent explosive disorder (19.6%) (Kessler et al., 2006). ADHD also shows high rates of comorbidity with other neurodevelopmental disorders and learning difficulties/ disabilities. One of the highest rates of comorbidity is with autism spectrum disorders, which can affect up to 50% of children, adolescents and adults with ADHD (Rommelse et al., 2010), while ADHD is also frequently comorbid with reading (8-39%) and mathematic (12-30%) disabilities (Barkley, 2006). ADHD is also negatively correlated with IQ, around 0.3 (Frazier et al., 2004). The association between ADHD and deficits in cognitive performance is considered in detail later in this thesis (section 1.6), as are the associations with temperament (section 1.7) and emotional lability (section 1.8).

1.4 QUANTITATIVE GENETICS

1.4.1 A definition of quantitative genetics

Quantitative genetics refers to a set of methods, including family, adoption and twin studies, used to partition phenotypic variance and covariance into genetic and environmental components (Plomin et al., 2008). This is accomplished by linking differential phenotypic resemblance between individuals to the functional effects of (differentially correlated) latent Genetic and Environmental factors. Strictly speaking, quantitative genetic studies focus specifically on continuously distributed phenotypes, however for the purposes of this thesis the term is used to define methodologies used to *estimate* genetic and environmental influences based on familial resemblance. Genetic components of variance can be additive, referring to a cumulative (additive) effect of alleles; or non-additive, referring to dominant or epistatic interactions between alleles (Plomin et al., 2008). Environmental components of variance can be shared, increasing the resemblance between related and unrelated individuals; or non-shared, reducing resemblance between individuals (Plomin et al., 2008). To partition variance and covariance into genetic and environmental components, quantitative genetic studies examine genetically related individuals to identify hereditary patterns. Specific genetic variants are not studied and accordingly this thesis makes a distinction between the terms *quantitative genetics* and *molecular genetics*, the latter of which refers to the study of genetic variants at the DNA level (see section 1.5).

1.4.2 Family studies

Family studies assess the resemblance between genetically-related individuals to estimate the extent to which a phenotype runs in families (Plomin et al., 2008). This method is based on coefficients of relatedness, which refer to the percentage of segregating alleles shared by common descent. A coefficient of relatedness is calculated as 0.5 to the power of the number of generational links. For example, the coefficient between first-degree relatives, such as father and son, would be 0.5^1, which equals 0.5; the coefficient between second-degree relatives, such grandfather and grandson, would be 0.5^2, which equals

0.25. It is therefore assumed that there will be greater resemblance between more closely related individuals if a phenotype is influenced by genes. However, because genetically-related family members who live together also share the same environment, a limitation of the family design is that it is unable to partition familial resemblance into genetic versus shared-environmental effects.

Family studies indicate an increased risk for the development of ADHD among the relatives of probands (Faraone et al., 2005b). A meta-analysis of six family studies indicated that 27% of the first-degree relatives of ADHD probands also met diagnostic criteria for ADHD, compared to just 6% in the family members of controls (Stawicki et al., 2006). This indicates that the risk of ADHD in the first-degree relatives of probands is almost five times greater than the risk within the general population, suggesting substantial familial transmission. The results of a recent, large-scale family study confirm this finding, also indicating a familial association between ADHD as a categorical diagnosis in probands and ADHD symptoms in their siblings with no evidence of diagnostic threshold effects (Chen et al., 2008). These findings suggest the same familial aetiology for ADHD as a clinical disorder and as a continuous trait.

Family studies indicate familial co-segregation of ADHD with other phenotypes. This includes externalising problems, such as substance use, oppositional-defiance, conduct problems and antisocial behaviour (Milberger et al., 1997, Petty et al., 2009, Faraone et al., 1997); internalising problems, such as anxiety and depression (Antshel et al., 2013, Biederman et al., 2012c, Faraone and Biederman, 1997); and autism spectrum disorder symptoms (Mulligan et al., 2009, Nijmeijer et al., 2009). Familial associations with emotional lability cognitive performance have also been identified and are discussed in detail in subsequent sections of this thesis (sections 1.6.3 and 1.8.4). These results suggest that many of the comorbidities associated with ADHD also run in the families of ADHD probands. However, the extent to which familial transmission and co-segregation reflects genetic and shared environmental effects cannot be determined via family studies alone.

1.4.3 Adoption studies

Adoption studies compare adoptees to their biological and adoptive relatives to examine resemblance for a phenotype (Plomin et al., 2008). Comparison in this manner allows the relative contributions of genes and the environment to be estimated: greater resemblance among the biological family suggests genetic contributions to phenotypic resemblance, since biological family members share genes but no environment with adoptees; greater resemblance among the adoptive family suggests a shared-environmental contribution to phenotypic resemblance, since the adoptive family share an environment with adoptees but are genetically unrelated. The ability to decompose variance into genetic and shared environmental components is an advantage of adoption over family designs.

There have been few adoption studies of ADHD. The most recent study to date compared 25 adopted children with ADHD to their 62 first-degree adoptive relatives, 101 non-adopted children with ADHD to their 310 biological relatives, and a control group of 50 non-adopted children without ADHD to their 153 biological relatives. Results indicated that only 6% of the adoptive parents of adopted ADHD probands fulfilled criteria for ADHD, compared with 18% of the biological parents of non-adopted probands. Similarly, only 8% of adoptive siblings met the diagnostic criteria for ADHD, compared with 31% of biological siblings. Rates of ADHD in the biological parents and siblings of controls were 3% and 6% respectively. This indicates significantly higher rates of ADHD in the biological family members of probands, suggesting genetic contributions to ADHD. Similar results were also reported in earlier adoption studies of ADHD (Alberts-Corush et al., 1986, Morrison and Stewart, 1973, Cantwell, 1975).

1.4.4 Univariate twin studies

Classical twin studies compare resemblance among identical and non-identical twins (reared together) to decompose phenotypic variance into genetic and environmental components (Plomin et al., 2008). As with family studies, twin studies make use of coefficients of relatedness to estimate genetic and environmental effects, based on the number of segregating alleles shared by

twins. Identical twins develop from a single zygote that forms two embryos during pregnancy, hence referred to as monozygotic (MZ). Consequently, the MZ twin coefficient of relatedness is 1.00, indicating that MZ twins share virtually all of their segregating alleles. Non-identical twins develop from two separately fertilized zygotes and are thus dizygotic (DZ). DZ twins therefore have a coefficient of relatedness of 0.50, the same as for other full siblings, indicating that they share on average 50% of their segregating alleles.

Based on the expected coefficients of relatedness, there should be greater resemblance among MZ than DZ twins for a phenotype that is genetic in origin, based on cross-twin within-trait correlations (Rijsdijk and Sham, 2002). Additive genetic components of variance are indicated when the cross-twin within-trait correlation for DZ twin pairs is around half of that found for MZ twin pairs, while non-additive genetic components are indicated when the DZ twin correlations are less than half of those for MZ twins. The sum of additive and non-additive components of variance gives rise to an estimate of broad-sense heritability. DZ twin correlations that are greater than half the MZ twin correlations indicate a role of the shared environment; while less than perfect correlations between twins from MZ or DZ pairs indicate a role of the non-shared environment. The non-shared environmental component of variance also subsumes any measurement error. The twin method, its assumptions and limitations are considered in detail in the methods section of this thesis (section 2.3).

Univariate twin studies of ADHD consistently estimate high heritability, around 70-80% (Faraone et al., 2005b). In a recent meta-analysis of 26 independent samples the heritability of ADHD was estimated at 70%, based on correlational data from 25,712 sibling pairs (Burt, 2009). Another recent meta-analysis estimated heritability of 73% for the symptoms of hyperactivity-impulsivity and 71% for the symptoms of inattention (Nikolas and Burt, 2010). The studies included in these meta-analyses primarily took a dimensional approach to assessing ADHD, in which the total variance in continuous ADHD symptom scores was decomposed into genetic and environmental components. However, an alternative approach is to examine concordance rates for categorically defined cases of ADHD in MZ and DZ pairs. This approach has also indicated greater concordance in MZ than DZ twin pairs, leading to

heritability estimates in the region of 50-80% (Goodman and Stevenson, 1989b, Lichtenstein et al., 2010, Thapar et al., 2000). This suggests that ADHD is heritable whether treated as a categorical diagnosis or continuous trait.

Studies utilising a Defries and Fulker (DF) extremes analysis approach (DeFries and Fulker, 1985, DeFries and Fulker, 1988) have also indicated similar levels of heritability. In the DF extremes design, proband twins are selected on the basis of affection status or extreme symptom scores for a phenotype. Proband scores for the phenotype are then used to predict symptom scores in their co-twins, based on a regression to the population mean. If the phenotype is influenced by genetic effects then the co-twin scores should regress back towards the population mean, but with the co-twin scores for DZ twins regressing back further than the scores for MZ twins. This pattern of results has been found in DF analyses of ADHD symptoms (Gjone et al., 1996, Larsson et al., 2012a, Levy et al., 1997, Stevenson, 1992).

In augmented DF analysis, using larger sample sizes, results additionally indicate that the heritability of extreme and sub-threshold ADHD symptoms is the same, and that the same genetic influences account for ADHD symptoms in the extreme and sub-threshold groups (Larsson et al., 2012a, Levy et al., 1997). This suggests that categorically defined cases of ADHD can be seen as representing the extreme end of a continuously distributed trait, with a common genetic liability operating across the continuum, indicating that ADHD is a quantitative trait (Plomin et al., 2009).

The heritability estimates obtained in twin studies of ADHD are often broad-sense, indicating an influence of both additive and non-additive genetic effects. In the meta-analysis by Burt (2009), around 26% of the total variance in ADHD was attributable to an additive genetic component whereas 44% of the variance was attributable to a non-additive genetic component. This indicates that non-additive genetic effects may account for a substantial proportion of the total heritability estimated for ADHD. However, in the subsequent meta-analysis by Nikolas and Burt (2010), significant non-additive genetic influences were found for symptoms of inattention only: 56% of the total variance in inattention symptoms was attributable to an additive genetic component and 15% to a non-

additive genetic component. This suggests that genetic non-additivity may be limited to the inattentive rather than hyperactive-impulsive domain. The meta-analysis by Nikolas and Burt (2010) further found that non-additive genetic effects were specific to parent and not teacher ratings of ADHD, indicating a rater difference in the source of genetic influences. Studies of self-rated ADHD symptoms have also failed to identify non-additive genetic effects. This pattern of results is consistent with a rater contrast effect that uniquely influences parental reports of ADHD symptoms, described in detail in section 1.4.6. It should be noted that genuine non-additive genetic effects are notoriously difficult to detect using the classical twin design, which lacks power even with large sample sizes (Keller et al., 2010, Rietveld et al., 2003).

Twin studies suggest that the environment makes only a negligible contribution to individual differences in the symptoms of ADHD, with minimal influences of the non-shared environment and virtually no evidence of shared environmental effects (Burt, 2009, Nikolas and Burt, 2010). This is in contrast to other psychiatric phenotypes, including other forms of externalised behaviours, for which shared environmental effects are usually found to account for 10-15% of the total phenotypic variation (Burt, 2009).

Although this pattern of results appears robust it is possible that shared environmental effects on ADHD are underestimated when using the classical twin design. This may be due to low power to detect shared-environmental effects, a confounding of shared-environmental and non-additive genetic effects, an overshadowing effect caused by contrast effects, or distributional issues leading to increased measurement error (Wood et al., 2010b). Re-analysis of the meta-analytic data presented by Burt (2009) indicated that low power and confounding due to either genetic non-additivity or contrast effects were unlikely to have accounted for the lack of shared environmental effects observed for ADHD (Burt, 2010). However, it is plausible that future analyses using less error-prone measures of ADHD symptoms, or extensions of the classical twin design such as extended twin-family studies (Keller et al., 2010), may find some evidence of shared environmental influences on ADHD. A greater role of the non-shared environment has also been found for different-teacher ratings and self-ratings of ADHD symptoms, discussed in detail in

section 1.4.6. Finally, it should be noted that these results does not preclude an important role for gene-environment interplay in the aetiology of ADHD (Rutter et al., 2006).

Another important finding to arise from univariate twin studies is in relation to aetiological sex differences. These are differences between males and females in either the source (qualitative sex differences) and/or magnitude (quantitative sex differences) of genetic and environmental effects, which can be tested via sex limitation twin models (see section 2.3.6). A recent review identified only a handful of twin studies reporting significant qualitative or quantitative sex differences in the aetiological influences on ADHD (Freitag et al., 2010), while the meta-analysis by Nikolas and Burt (2010) generally supported the conclusion that aetiological influences on hyperactivity-impulsivity and inattention did not differ across sex. While some studies of ADHD have identified significant sex differences in phenotypic variances (e.g. Price et al, 2005), referred to as scalar sex differences, these can be accounted for in twin modelling and do not indicate aetiological differences by sex.

1.4.5 Multivariate twin studies

Multivariate extensions of the twin method have been used to examine genetic and environmental contributions to phenotypic covariance, testing the extent to which the same aetiological factors are associated across different phenotypes. Whereas univariate analyses only examine resemblance of the same trait within MZ and DZ twin pairs, multivariate analyses also examine the resemblance of different traits within pairs. Such studies have furthered understanding of the aetiology of ADHD in a number of ways.

First, multivariate twin studies have demonstrated that the ADHD symptom dimensions of hyperactivity-impulsivity and inattention share much of their aetiology, building on evidence of a substantial but imperfect phenotypic association (Toplak et al., 2009, Toplak et al., 2012). For example, analyses in childhood (McLoughlin et al., 2007), adolescence (Greven et al., 2011c) and adulthood (Larsson et al., 2013) have identified phenotypic correlations between hyperactivity-impulsivity and inattention of around 0.60 to 0.70, with

genetic correlations (r_G) also of around 0.6 to 0.7. The proportion of the phenotypic correlation accounted for by shared genetic influences (i.e. the bivariate heritability) is typically around 70%, suggesting that genetic influences account for most of the cross-sectional covariation between symptoms of hyperactivity-impulsivity and inattention at different developmental stages. The fact that not all aetiological influences are shared indicates that there is also genetic heterogeneity.

Second, multivariate twin studies have examined the stability and change in genetic and environmental influences on ADHD over time. Longitudinal studies indicate moderate stability of total ADHD symptoms across early and middle childhood (Kuntsi et al., 2005b, Price et al., 2005), primarily accounted for by stable genetic effects. Other studies have revealed somewhat higher stability from middle childhood through to adolescence and adulthood, also due to stable genetic influences but with evidence of newly emerging genetic effects accounting for changes in symptoms over time (Chang et al., 2013, Larsson et al., 2004, Van Den Berg et al., 2006). These studies identified modest effects of the non-shared environment on both stability and change in the symptoms of ADHD and are therefore somewhat consistent with a recent auto-regressive twin study, which found that stability in ADHD symptoms from childhood through to older adulthood was due to a combination of genetic and environmental effects (Kan et al., 2013).

Studies examining the two dimensions of ADHD separately have revealed a similar pattern of results, indicating predominantly shared genetic influences for hyperactivity-impulsivity and inattention over time, but with some unique genetic influences across time points and symptom dimensions (Greven et al., 2011a, Larsson et al., 2006, Nadder et al., 2002, Rietveld et al., 2004). Recent research additionally suggests that there may be a unidirectional association between the two dimensions, with childhood hyperactive-impulsive symptoms predicting later inattention and not vice versa (Greven et al., 2011a). Taken together, these multivariate studies indicate that ADHD is substantially influenced by genetic factors across development, with genetic stability but also innovation, and with a lesser role of the non-shared environment.

Third, multivariate studies have identified genetic associations between ADHD and a number of co-occurring phenotypes. This includes symptoms of depression and negative emotionality (Cole et al., 2009, Singh and Waldman, 2010); borderline personality disorder symptoms (Distel et al., 2011); externalising behaviours such as oppositional-defiance, conduct problems and substance use (Chang et al., 2012, Nadder et al., 2002, Thapar et al., 2000, Tuvblad et al., 2005, Tuvblad et al., 2009, Wood et al., 2009a, Young et al., 2009b, Young et al., 2000); autism spectrum disorder symptoms (Lichtenstein et al., 2010, Reiersen et al., 2008, Rommelse et al., 2010, Ronald et al., 2010, Ronald et al., 2008); poor motor control (Martin et al., 2006); reading disability symptoms (Greven et al., 2011b, Greven et al., 2012, Paloyelis et al., 2010b, Willcutt et al., 2010, Willcutt et al., 2007); and low IQ (Kuntsi et al., 2004, Polderman et al., 2007, Polderman et al., 2006). Quantitative genetic associations of ADHD symptoms with other phenotypes including cognitive performance, temperament and the symptoms of emotional lability are considered in subsequent sections of this thesis (sections 1.6 1.7 and 1.8).

Although this list is non-exhaustive, findings are consistent with the range of comorbidities reported in individuals diagnosed with ADHD (see section 1.3.5). This suggests that many of the phenotypes that occur alongside ADHD may do so because of shared genetic effects. Some of the phenotypes linked to ADHD have shown specificity in their genetic associations with the two ADHD symptom dimensions; for example poor reading ability appears uniquely associated with inattentive symptoms (Greven et al., 2011b, Paloyelis et al., 2010b), while oppositional-defiance is uniquely associated with the hyperactive-impulsive dimension (Wood et al., 2009b). This provides additional information regarding the heterogeneity of ADHD, highlighting the fact that hyperactive-impulsive and inattentive symptoms are imperfectly related. This suggests that future studies may benefit from examining the two dimensions separately when exploring the aetiological associations of co-occurring phenotypes with ADHD.

1.4.6 Rater effects

Rater effects are an important issue that can influence or bias the heritability estimates derived from twin studies. Understanding such influences is imperative in order to accurately characterise the aetiology of ADHD, with knock-on effects for neurobiological and molecular genetic research. Here, the term *rater effect* is used to refer to three key concepts in the twin literature on ADHD: contrast effects, rater differences in heritability estimates and rater agreement.

Contrast effects refer to either a competitive (negative) sibling interaction, in which one twin's behaviour influences that of the co-twin; or a rater effect that occurs when the informant completing rating scales directly contrasts the behaviours of each twin from a pair (Neale and Maes, 2004). The contrast effect therefore acts to reduce twin similarity, but with a greater impact on DZ than MZ twins. If not accounted for during genetic modelling, contrast effects result in inflated estimates of heritability. The presence of contrast effects is indicated by low, sometimes negative, DZ cross-twin within-trait correlations in tandem with significantly greater phenotypic variances for DZ than MZ twins. It is this variance difference that distinguishes contrast effects from non-additive genetic effects, which are also indicated by low DZ correlations.

Contrast effects have been identified in numerous twin studies of ADHD (see Rietveld et al., 2003; Stevenson et al., 2005; and Freitag et al, 2010) and it is argued that undetected contrast effects may account for some of the non-additive genetic influences found for ADHD (Wood et al., 2010b). The contrast effect appears to be specific to parental reports of ADHD symptoms, suggesting a form of rater bias as opposed to genuine behavioural interaction (Simonoff et al., 1998). Indeed, the effect is not reported for teacher or self-ratings of ADHD, although teacher ratings may be subject to their own form of bias whereby same-teacher ratings are more highly correlated than different teacher-ratings (Simonoff et al., 1998). Research indicates that the contrast effect may be more pronounced when using short rating scales (e.g. the 5-item SDQ 5 hyperactivity scale or the 3-item Rutter A scale) to assess the symptoms of ADHD (e.g. Price et al, 2005), suggesting that longer rating scales may provide more objective

measures of behaviour. The contrast effect is also more pronounced in smaller families (Pinto et al., 2012), suggesting that parents directly contrast the behaviour of twins when they lack other same-age children against which to judge behavioural norms. This is consistent with the view that teacher ratings are robust against contrast effects because teachers have a wide experience of same-age children against which to compare the normality of twins' behaviours (Hartman et al., 2007, Simonoff et al., 1998).

The contrast effect literature identifies one obvious distinction between parent and other-informant ratings of ADHD symptoms; namely that heritability estimates derived from parent ratings of ADHD may be uniquely biased by contrast effects. This is consistent with the finding of significant non-additive genetic effects for parent but not teacher ratings of ADHD symptoms (Nikolas and Burt, 2010). However, another potential rater effect concerns differences in the magnitude of broad-sense heritability estimates derived from different informant ratings of ADHD.

In the recent meta-analysis by Nikolas and Burt (2010) the heritability of hyperactivity-impulsivity and inattention was highly similar when symptoms were rated by parents (74% and 72%) and teachers (77% respectively). This suggests that the different informant ratings yield similar estimates of heritability. Yet individual studies indicate that the heritability of teacher-rated ADHD symptoms is often lower (Kuntsi and Stevenson, 2001, Thapar et al., 2001), particularly when different teachers rate the behaviours of each twin from a pair (Derks et al., 2006, Hartman et al., 2007, Saudino et al., 2005, Simonoff et al., 1998). These results point towards greater similarity in the ratings of behaviour for same than different teachers. Simonoff et al (1998) interpreted this as evidence of bias, with either twin confusion or correlated errors leading to inflated heritability estimates for same-teacher ratings of ADHD symptoms. In contrast, Derks et al (2006) argued that the lower heritability of different-teacher ratings could reflect genuine behavioural differences in the interactions of twins with different teachers. Yet another explanation is one of increased measurement error, which occurs when different teachers rate each twin from a pair, leading to lower heritability estimates (Hartman et al., 2007). This is a plausible explanation since different informant ratings of ADHD are thought to

be less reliable due to low inter-rater agreement. This increases measurement error and places a ceiling limit on estimates of heritability (Plomin et al., 2008).

Consistent with the data for different-teachers, self-ratings of ADHD symptoms also yield lower estimates of heritability. One of the initial studies on this topic estimated zero heritability for self-rated symptoms of ADHD, finding instead that the non-shared environment and/or measurement error accounted for the majority (71%) of phenotypic variance (Martin et al., 2002). The remaining variance was accounted for by the shared environment. Subsequent studies have failed to replicate this result, but have consistently estimated heritabilities within the region of 30-50%. This is true of studies using self-ratings obtained during adolescence, including via questionnaire and interview-based measures (Chang et al., 2013, Ehringer et al., 2006a, Kan et al., 2013, Young et al., 2009b, Young et al., 2000); of retrospective self-ratings of childhood ADHD symptoms made during adulthood (Haberstick et al., 2008, Schultz et al., 2006); and of self-ratings obtained prospectively in adulthood (Boomsma et al., 2010, Chang et al., 2013, Kan et al., 2013, Larsson et al., 2012b, Van Den Berg et al., 2006). This suggests that the heritability estimated for self-ratings is consistently lower than for parent or same-teacher ratings of ADHD symptoms.

Because self-ratings are most commonly used in adulthood (Asherson, 2005), one initial interpretation of these results was of a developmental decline in the heritability of ADHD (Boomsma et al., 2010, Van Den Berg et al., 2006). However, the results of recent longitudinal analyses dispute this conclusion, with one study indicating that a decline in the heritability of ADHD symptoms coincides with a switch from parent ratings to self-ratings of ADHD (Kan et al., 2013), and another indicating that the heritability of ADHD symptoms is consistently high based on latent factors derived from parent and self-ratings (Chang et al., 2013). This strongly suggests that the lower heritability reported in some adult studies of ADHD can be attributed to the use of self-ratings rather than a genuine developmental trend.

Despite different heritabilities, there is modest agreement between the multiple informant ratings of ADHD symptoms. Rater agreement has been assessed via multivariate twin studies examining the extent to which common aetiological

factors account for the variance in different informant ratings of ADHD. Common genetic and environmental influences indicate that different informants are rating similar aspects of behaviour, while rater-specific genetic influences indicate that different informants rate unique aspects of behaviour; rater specific environmental effects can reflect either rater bias via the shared-environmental component, or measurement error via the non-shared environment (Hewitt et al., 1992). Most studies of rater agreement in ADHD have compared parent and teacher ratings, finding shared but also specific aetiological influences across different informant ratings (Derks et al., 2006, Hartman et al., 2007, Martin et al., 2002, Nadder et al., 2002, Thapar et al., 2000). This suggests that there is a common, pervasive view of ADHD-related behaviours influenced by a common set of genes, in addition to unique components of behaviour assessed by different informants and with unique but valid genetic influences on behaviour.

Only one study has examined the association between parent and teacher ratings across two dimensions of ADHD, finding that different informants rated somewhat different aspects of hyperactive-impulsive and inattentive behaviours (McLoughlin et al, 2011). Similarly, only one study has examined the association between parent and self-ratings of ADHD symptoms, which was due largely to overlapping genetic influences across development (Chang et al., 2013). These results indicate modest rater agreement that is largely attributable to common genetic influences on behaviours; however the extent of the association between parent, teacher and self-ratings of ADHD symptoms remains to be explored. Nonetheless, the available evidence suggests that neurobiological and molecular genetic research may benefit from taking a pervasive, multi-rater view of ADHD-related behaviours in order to tap into a more heritable phenotype that more closely resembles the clinical disorder (Stevenson et al., 2005).

1.5 MOLECULAR GENETICS

1.5.1 A definition of molecular genetics

Molecular genetic studies of psychiatric disorders generally refer to the study of specific genetic variants at the DNA level and their association with clinical or behavioural phenotypes (Plomin et al., 2008); as well as the molecular mechanisms that mediate such gene-phenotype relationships. As in quantitative genetics, a phenotype can be categorical, where the goal is to see whether genetic variants are associated with affection status; or continuous, testing for a linear association between genetic variants and quantitative trait scores. Under the latter approach genetic variants are referred to as quantitative trait loci and are generally assumed to have an additive effect on disease status (Plomin et al., 2008). This is based on the observation that many clinical phenotypes can be seen as the extreme manifestation of quantitative, polygenic traits influenced by additive genetic effects (Plomin et al., 2009). In this sense, quantitative and molecular genetics research methods can be used to address complementary research questions regarding the aetiology of a phenotype.

Molecular genetic analyses have examined ADHD as both a categorical and continuous phenotype, with the largest most statistically powerful datasets so far using ADHD case-control designs. The earliest studies took a candidate gene approach, testing risk alleles from specific genes for association with ADHD. Subsequent studies have taken a genome-wide approach, using affected sibling pair linkage designs initially, and more recently genome wide association studies (GWAS). Other recent analyses have explored the polygenic basis of ADHD and the role of rare copy number variants.

1.5.2 Candidate gene association

Candidate gene studies examine the association of “risk” alleles for a specific gene with a phenotype, based on a-priori hypotheses (Plomin et al., 2008). Two main methods of candidate gene association study are used. The first method is population-based association, testing for a relationship between potential risk alleles and a phenotype in unrelated individuals. Analyses either examine the

phenotype at a categorical level, comparing the number of candidate risk alleles in cases versus controls, or examine the linear association between number of alleles and a continuous phenotype score. However, one limitation is that results may be biased by population stratification, in which systematic differences in allele frequencies in sub-populations account for the associations observed (Benyamin et al., 2009).

The second method is family-based association. This method examines whether there is significant over-transmission of candidate risk alleles from parents to their affected offspring, or whether over-transmission of alleles is associated with higher continuous phenotypic scores. Such family-based designs are advantageous since they are robust to the effects of population stratification, but lack power compared to well-designed case-control studies (Benyamin et al., 2009). Both methods have been used in the study of ADHD.

The first candidate gene studies of ADHD tested for associations of dopaminergic genes with ADHD affection status, including the dopaminergic receptor D4 gene (DRD4), the dopamine transporter gene (DAT1), and later the dopamine receptor D5 gene (see Asherson and Gurling, 2012, for a review). These studies identified strong associations with ADHD affection status, notably for the 7-repeat of a variable number tandem repeat (VNTR) within DRD4 and of a microsatellite marker within DRD5, which both reached the genome-wide significance level ($p < 5*10^{-8}$, Dudbridge and Gusnanto, 2008) in a well conducted meta-analysis (Li et al., 2006).

Subsequent studies have continued to assess the association between dopaminergic genes and ADHD, in addition to associations of other genes from systems of interest. However, a meta-analysis of candidate gene studies (both population and family-based studies) identified only five genes significantly associated with child and adolescent ADHD (Gizer et al., 2009). These included DAT1, DRD4 and DRD5, in addition to the serotonin transporter gene (5HTT) and synaptosomal protein 25 gene (SNAP25). DAT1, DRD4, DRD5, 5HTT and SNAP25 also showed evidence of significant heterogeneity in their effect sizes across studies, as did the dopamine beta hydroxylase gene (DBH), adrenergic receptor 2A gene (ADRA2A), tryptophan hydroxylase 2 gene (TPH2) and the

monoamine oxidase A gene (MAOA). Future association studies may therefore prove more successful if they focus on more homogeneous subsamples of children and adolescents with ADHD (Gizer et al., 2009). These results consistently implicate monoamine system genes as risk factors for ADHD, but with relatively weak effect sizes for most markers studied (odds ratios from meta-analysis no higher than 1.33). Furthermore, even following meta-analysis, most of these findings are far from genome-wide significant levels and it therefore remains feasible that the current larger scale studies will fail to replicate many of these initial results.

Candidate gene studies of adult ADHD have similarly focused on the monoamine system and have been recently reviewed (Franke et al., 2012), the main results of which are reported here. Of the 46 population and/or family-based association studies identified, most examined the genes DAT1 and DRD4. DAT1 was not consistently associated with ADHD in adults across studies, although some studies identified significant associations of the 9-repeat from the 3' untranslated region (UTR) of DAT1 with adult ADHD. This is in contrast to the earlier findings of association between the 10-repeat and child and adolescent ADHD; and could indicate that the 9-repeat indexes a severe, persistent form of the disorder (Franke et al., 2012). Analyses of DRD4 have also been inconsistent, with only weak evidence of association between adult ADHD and the DRD4 7-repeat, but with one longitudinal study linking the 7-repeat to persistent ADHD. Other studies have identified modest associations of adult ADHD with DRD5, DBH, TPH2, the catechol-O-methyltransferase gene (COMT) and the serotonin receptor 2A gene (HTR2A) but have failed to identify convincing associations with 5HTT, the adrenergic receptor genes 2A and 2C, or the noradrenergic transporter gene (NET). More recent studies of adult ADHD have examined associations with genes outside of the monoamine system, identifying significant associations with the brain-specific angiogenesis inhibitor 1-associated protein 2 gene (BAIAP2), the circadian locomotor output cycles kaput gene (CLOCK) and the nitric oxide synthase 1 gene (NOS1).

Candidate gene research has also sought to identify quantitative trait loci associated with continuous ADHD symptom scores. A recent family-based study of children, adolescents and young adults from a population twin register

identified significant associations of higher total ADHD symptom scores with the DRD4 4-repeat and the DAT1 10-repeat alleles (Bidwell et al., 2011). This analysis additionally indicated that the DAT1 10-repeat was more strongly related to inattentive than hyperactive-impulsive symptoms of ADHD. Another study found that the DRD4 7-repeat was significantly associated with weighted symptoms of inattention generated using principal components analyses to maximise trait heritability (Lasky-Su et al., 2008a). However, a large study of 1,148 children from a population-based twin register failed to identify significant candidate gene associations of HTR2A, COMT, TPH2 and the brain derived neurotrophic factor gene (BDNF) with a latent factor that accounted for stability of attention problems at 3, 7, 10 and 12 years of age (van Beijsterveldt et al., 2011).

In summary, the most consistent results to emerge from candidate gene studies of ADHD are for dopaminergic genes, in particular the 7-repeat allele of the DRD4 gene. This is true of studies in children, adolescents and adults, and of studies examining ADHD as either a categorical or continuous phenotype. However a major limitation of the ADHD candidate gene studies is that effect sizes are small and that findings have typically failed to reach anywhere near the level of genome-wide significance. Therefore the findings only account for a very small proportion of the total heritability estimated for ADHD. A second limitation is that candidate gene studies are hypothesis driven and only examine one or few known variants at a time; and it may well be that many prior hypotheses are wrong. An alternative, more exploratory approach is to conduct genome-wide analyses, testing markers from multiple different genes and control regions across the entire human genome for their association with ADHD. This has greater the potential to identify novel genetic associations.

1.5.3 Genome-wide association

Genome-wide association studies (GWAS) examine the association of common genetic variants from throughout the genome with a categorical or continuous phenotype (Plomin et al., 2008). This method is based on the assumption that many common genetic variants confer a small, additive risk for a phenotype of interest. Like candidate-gene association studies, GWAS use population or

family-based methodologies, testing variants for association with either categorical or continuous phenotypes. However unlike candidate gene studies GWAS can be performed in an exploratory, hypothesis-free manner. To account for multiple testing a stringent significance threshold is employed, calculated as $p < 5*10^{-8}$ (Dudbridge and Gusnanto, 2008).

Most GWAS to date have examined ADHD as a categorical disorder in children and adolescents, but with limited success. The first published studies failed to identify results significant at the adjusted threshold of $p < 5*10^{-8}$ (Mick et al., 2010, Neale et al., 2008, Neale et al., 2010a), as did a meta-analysis of ADHD GWAS published in 2010 (Neale et al., 2010b) using the largest sample size available at that time (N = 5,415 individuals). More recent analysis has also failed to identify genome-wide significant associations with child and adolescent ADHD (Hinney et al., 2011), as did prior analysis of continuous ADHD symptom scores in children and adolescents (Lasky-Su et al., 2008b). The only published GWAS of adult ADHD similarly failed to identify significant effects (Lesch et al., 2008), as did a recent GWAS using child and adolescent data from a large Chinese Han population (Yang et al., 2013).

The lack of significant GWAS results is not specific to ADHD and until recently has characterised most genome-wide analyses conducted using psychiatric phenotypes. Putative reasons for this so-called *"missing heritability"* are that common variants interact in a dominant and/or epistatic fashion, that common variants interact with the environment in ways that are poorly understood and measured, that within-sample heterogeneity reduces the phenotypic variance explained by genes, and that common genetic variants confer only a small risk for complex disorders (Maher, 2008, Manolio et al., 2009). Of these reasons, the small effect size of common variants is considered important since it means existing studies are likely underpowered to detect genome-wide significant effects.

To resolve the issue of low power, the Psychiatric Genomics Consortium (PGC) was established in 2007 to facilitate the pooling of international genomic data (Sullivan, 2010). This has led to a gradual increase in the available samples for genomic studies of the psychiatric disorders ADHD, autism, bipolar disorder,

depression and schizophrenia. In the most recent mega-analysis of schizophrenia a discovery GWAS using a sample of 21,856 individuals and a replication GWAS using a sample of 29,839 individuals identified associations with seven loci at the genome-wide significant level (Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011). This suggests that larger samples are likely to lead to significant GWAS findings for ADHD.
A mega-analysis of ADHD has yet to be published using all available PGC ADHD data; however a recent cross-disorder mega-analysis identified three variants significantly associated with ADHD and the other PGC disorders at a genome-wide significant level (Smoller et al., 2013). Two of these markers were located close to multiple genes, however one marker was located close to a single gene involved in brain-based calcium channel activity, the calcium channel voltage-dependent beta 2 subunit (CACNB2). This shows that common genes may confer risk for multiple psychiatric disorders. It also suggests that significant genome-wide associations for ADHD will likely be identified with larger samples.

1.5.4 Polygenic association

Polygenic analyses have also been applied to genome-wide association data to test whether multiple genetic variants are associated with a phenotype en-masse. The primary assumption underlying this approach is that many common genetic variants confer a small, additive risk for phenotype affection status or for the severity of continuous phenotype symptoms. Polygenic approaches therefore allow meaningful information to be extracted from existing, underpowered GWAS samples by examining multiple variants *en-masse*. Three main polygenic methods are considered: gene pathway analysis, the profile (allele) score method, and genome-wide complex traits analysis (GCTA).

Gene pathway analysis examines the association of a phenotype with genes that work together within functional networks. Genes for inclusion in a pathway are identified based on previous associations reported within the literature and via bioinformatics analysis used to extract meaningful information from existing data. A recent study of ADHD examined 85 of the top-ranked single nucleotide polymorphisms (SNPs) identified from five previous GWAS (Poelmans et al.,

2011). These SNPs were significantly associated with ADHD and bioinformatic analyses indicated that 45 genes fit into a neurodevelopmental network associated with neurite outgrowth. Subsequent research from the same group has replicated and extended this finding, showing significant associations of serotonergic, dopaminergic and neurite outgrowth gene networks with ADHD (Bralten and Franke, unpublished data). Pathway analysis conducted using Chinese data has also identified a network of 16 proteins involved in cell adhesion, synaptic formation and neuronal plasticity that were significantly associated with ADHD (Yang et al., 2013). These findings so far indicate that systems of genes involved in both neurotransmission and neuronal development may confer a risk for ADHD.

The profile score method tests for en-masse associations of genetic variants with a phenotype. A profile score is generated using all risk alleles associated with a phenotype at specified significance threshold (e.g. $p < .05$) in a discovery dataset. The number of reference ("risk") alleles carried by each individual within an independent, target dataset is then calculated and used to predict the phenotype of interest (Evans et al., 2009). The method is described in detail later in this thesis (section 2.4.2). The first application of the profile score method to a psychiatric disorder was for schizophrenia (Purcell et al., 2009). All SNPs (n = 37,655) associated with schizophrenia at the threshold $p < 0.5$ in a discovery sample (N = 3,818) were predictive of schizophrenia in an independent target sample (N = 3,091), explaining roughly 3% of the total variance in schizophrenia affection status. Subsequent analyses using a larger sample (discovery sample N = 15,492, target sample N = 6,482) increased the total variance explained to approximately 6%. This further highlights the potential value of increasing the sample sizes for genetic studies of ADHD.

To date three published profile score analyses have examined ADHD. The first was a cross-disorder study using the PGC dataset to examine associations between ADHD, autism, bipolar disorder, depression and schizophrenia (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). Significant cross-disorder associations were found for all phenotypes apart from ADHD. However a second, smaller study identified significant associations of profile scores from schizophrenia and bipolar disorder discovery sets with ADHD,

explaining up to 0.6% of the variance in ADHD affection status (Hamshere et al., 2013b). The results across studies suggest that common genetic variation may confer risk for multiple disorders, perhaps linking schizophrenia and bipolar disorder to ADHD, although further replications are required.

The third published study (Hamshere et al., 2013a) generated a profile score using data from the ADHD GWAS meta-analysis (Neale et al., 2010b), using reference alleles from all SNPs associated with ADHD affection status at the threshold $p < 0.5$. The score explained around 0.1% of the variance in ADHD affection status in an independent target set comprising 452 ADHD children and 5,081 controls. This signal was enriched in cases from the target set with high levels of conduct problems, in which it accounted for around 1.1% of the variance in ADHD. There was also a significant continuous association between the profile score and greater symptom scores for conduct problems, suggesting a common genetic liability for conduct problems and ADHD. An advantage of this study compared to the other two is that it was able to demonstrate polygenic inheritance for ADHD by generating and testing a polygenic score in ADHD case/control samples. However, the polygenic basis of ADHD is still poorly understood. One reason is that replication studies are required, including those that generate multiple thresholds of profile score. Another reason is that analyses have yet to determine whether a polygenic score for ADHD affection status can also predict continuous ADHD symptom scores. This would provide a direct test of the quantitative trait hypothesis of ADHD underlying much genetic research.

The GCTA method is used to estimate the heritability of a phenotype as a function of the variance explained by all autosomal SNPs. The method was first applied to the study of human height, indicating that all SNPs (n = 294,831) in a genome-wide study of 3,925 individuals accounted for 45% of the total variance in height (Yang et al., 2010). This estimate increased to 84% when correcting the model for SNPs in incomplete linkage disequilibrium, in line with the 80% heritability estimate derived from twin studies (Macgregor et al., 2006) and much higher than the 5% variance explained by GWAS (Visscher, 2008). The GCTA method can therefore be used to derive estimates of heritability based on all available SNPs in genome-wide datasets (i.e. SNP-wide heritability, SNP-h^2).

Two GCTA studies of ADHD have been conducted to date. The first was part of a PGC cross-disorder initiative, which estimated SNP-h^2 of 28% for ADHD affection status (Cross-Disorder Group of the Psychiatric Genomics Consortium, in press). A bivariate application of GCTA additionally indicated a genetic correlation between ADHD and depression of 0.32. The main implication of these findings is that a significant proportion of the variance in ADHD affection status is accounted for by common SNPs tagged by genome-wide arrays. This indicates that ADHD as a disorder is heritable, but with a substantial proportion of missing heritability when compared to the results of twin research.

The second GCTA study examined ADHD symptoms as continuous traits and identified a different pattern of results (Trzaskowski et al., in press). This study examined children aged approximately 12 years from the Twins Early Development Study (TEDS) and failed to estimate significant SNP-h^2 (± standard error) for parent (SNP-h^2 = 0 ± 0.12), teacher (SNP-h^2 = 0.05 ± 0.15) and self-ratings (SNP-h^2 = 0 ± 0.12) of ADHD using the Strengths and Difficulties Questionnaire (SDQ) hyperactivity scale, or for parent ratings of ADHD using Conners Parent Rating Scales (total ADHD SNP-h^2 = 0 ± 0.12, hyperactivity-impulsivity SNP-h^2 = 0.06 ± 0.12, inattention SNP-h^2 = 0 ± 0.12). These results were unlikely to be caused by genotyping errors, since SNP-h^2 estimates were around 40% for height and weight and 25% for measures of cognitive performance in the same sample. One conclusion was that these results could arise as a result of greater non-additivity for ADHD symptoms (Trzaskowski et al., in press). Whether these conflicting results indicate a genuine difference in the aetiology of ADHD between clinical and community samples remains unclear.

1.5.5 Rare variants

If common genetic variants account for a smaller proportion of the risk ADHD than had previously been thought, it is possible that there may be a greater role for rare variants with moderate to large effects (Manolio et al., 2009). Rare variants are usually defined as those with a minor allele frequency (MAF) of less

than 1% in the population. One method for exploring the role of rare variants of more moderate to large effect sizes has been the study of copy number variants (CNVs), which are duplications or deletions occurring across long stretches of DNA (Plomin et al., 2008).

In ADHD (and other neurodevelopmental disorders) the evidence for aetiologically significant CNVs is accumulating. The first study on this topic identified 222 inherited CNVs among ADHD probands and their parents, but was unable to identify significant case/control differences (Elia et al., 2010). However they found a significant increase in CNVs that also occurred in both schizophrenia and autism. A similar pattern of results was also reported in a study of severe ADHD children, in which identified CNVs were not recurrent across ADHD cases (Lesch et al., 2011). Nonetheless, subsequent studies have identified more robust findings, including duplications on chromosome 16p13.11 (Williams et al., 2010), duplications on chromosome 15q13.3 (Stergiakouli et al., 2012, Williams et al., 2012), enrichment of CNV deletions affecting metabotropic glutamate receptor genes on chromosomes 3, 7 and 11 (Elia et al., 2012), and deletions and duplications on chromosome 6 at the Parkinson protein 2 gene (PARK2) locus (Jarick et al., 2012). Several of the regions harbouring CNVs in ADHD also confer risk for other neurodevelopmental phenotypes, including low IQ, schizophrenia and autism and Tourette syndrome (Elia et al., 2010, Williams et al., 2012, Williams et al., 2010). This suggests that rare CNV duplications and deletions may represent more general risk factors for a range of neurodevelopmental disorders, a finding somewhat confirmed in another study that found no differences in ADHD symptom severity between CNV carriers and non-carriers, but significant differences in levels of intellectual disability (Langley et al., 2011).

1.5.5 Genome-wide linkage

Linkage studies examine the association of a phenotype with large chromosomal regions spanning many, sometimes thousands, of genes (Asherson and Gurling, 2012). One potential advantage compared to association studies is that linkage can be found that results from multiple different allelic variants of a gene, allowing for allelic heterogeneity, whereas

association studies test one specific risk allele or haplotype. Nevertheless, for the purposes of identifying genes for complex disorders, such as ADHD, linkage methods are in most cases underpowered because only relatively large genetic effects can be detected.

A meta-analysis of seven genome-wide linkage scans, predominantly of child and adolescent ADHD, found only one region on chromosome 16, between 16q21 and 16q24, that was significantly linked with ADHD, and identified suggestive linkage with ten additional regions on chromosomes 5, 6, 7, 8, 9, 15, 16 and 17 (Zhou et al., 2008). Linkage with the region spanning 16q21 to 16q24 is considered interesting since it houses the Cadherin 13 gene (CDH13), which showed nominal associations with child and adult ADHD in GWAS (Lasky-Su et al., 2008b, Lesch et al., 2008; as discussed in Asherson and Gurling, 2012). No genome-wide linkage scans have been conducted exclusively for adult ADHD (Franke et al., 2012), however analysis of children and adults with ADHD in large Columbian pedigrees identified a region of linkage on chromosome 4q13 that led to the discovery of the association between ADHD and the latrophilin 3 gene (LPHN3) (Arcos-Burgos et al., 2010). This last finding shows that in some cases, particularly in large genetically homogenous pedigrees, it is possible to detect some genes using linkage approaches.

1.6 COGNITIVE PERFORMANCE

1.6.1 Cognitive theories of ADHD

ADHD is a neurodevelopmental disorder and has accordingly been linked to a range of deficits in cognitive functioning and neuropsychological performance (hereafter referred to as cognitive performance deficits). This is in addition to the phenotypic and genetic associations observed between ADHD and low IQ (see sections 1.3.2 and 1.4.5). A recent review identified four major theories regarding cognitive performance deficits in ADHD that have sought to account for the neurocognitive basis of hyperactive-impulsive and inattentive behaviours (Johnson et al., 2009).

One major theory is of an executive functioning deficit in ADHD (Barkley, 1997, Pennington and Ozonoff, 1996). Executive functions are higher-order cognitive processes involved in decision-making and problem solving, including planning, sequencing, reasoning, vigilance, working memory and inhibition. These processes regulate lower-level cognitive functions, such as language, perception, explicit memory, learning and action (Johnson et al., 2009). This is a top-down model, in which higher order processes lead to lower-level processes and to the manifestation of behaviours. Executive functioning is thought to involve neural connectivity in the frontal lobe, in particular the prefrontal cortex, in addition to secondary connectivity within the thalamus and basal ganglia (Willcutt et al., 2005). This implicates both cortical and subcortical brain regions in the development of ADHD.

A number of executive dysfunctions have been reported in ADHD, although meta-analyses suggest that the most consistent case/control differences are found for measures of vigilance (i.e. the ability to sustain attention over time), working memory (i.e. the ability to hold and manipulate transitory information), planning (i.e. forethought towards achieving a desired goal) and response inhibition (i.e. the ability to withhold a pre-potent response) (Pauli-Pott and Becker, 2011, Willcutt et al., 2005). One specific hypothesis argues that poor inhibition represents a core deficit in ADHD, responsible for other cognitive and behavioural symptoms (Barkley, 1997). This theory postulates that inhibitory control has a top-down effect, regulating four executive functions (working memory, self-regulation, internalisation of speech and reconstitution of behaviour) and the inhibition of behaviour (Barkley, 1997). This theory is somewhat supported by meta-analytic data, in which measures of response inhibition showed the most consistent associations with ADHD. However, the medium effect sizes reported in meta-analysis (Cohen's d = 0.46-0.69) suggest that executive function deficits, including response inhibition, are insufficient to account for all of the variance observed in ADHD (Willcutt et al., 2005).

A second major theory is of suboptimal state regulation in ADHD (Johnson et al., 2009). State regulation has been described in the context of a cognitive-energetic model (CEM), which argues that information processing is determined via interplay at three levels: computational mechanisms of attention, energetic

mechanisms, and executive functions (Sergeant, 2000, Sergeant, 2005). The CEM thus incorporates both top-down and bottom-up cognitive processes linked to cortical and subcortical brain regions including the hippocampus, amygdala, basal ganglia, striatum and pre-frontal cortex (Sergeant, 2005, Sergeant et al., 2003). According to the CEM, state regulation difficulties in ADHD arise as a result of a failure to optimise energetic mechanisms of arousal and activation. Arousal refers to time-locked, phasic responding and is influenced by the intensity and novelty of stimuli. Activation refers to readiness to respond and is influenced by preparation and alertness. These mechanisms are contingent on a third mechanism, effort, which is required in order to meet task demands and to counteract deficiencies in arousal or activation. According to the CEM, executive function provides overall (top-down) control for the supply of effort to activation and arousal states, meaning that the model can account for the role of both regulatory and executive processes in ADHD.

According to the CEM, optimal task performance occurs when regulatory states of arousal and activation are optimised; yet in individuals with ADHD such optimal states are not consistently achieved. Evidence of sub-optimal state regulation in ADHD has come from studies of intra-individual variability, recently reviewed by Kuntsi and Klein (2012). Intra-individual variability refers to within-individual fluctuations in performance, typically measured as reaction time variability (RTV) during cognitive performance tasks. In conditions with slow event rates (i.e. slow presentation of stimuli) research consistently indicates greater RTV in individuals with ADHD than in controls, with additional evidence of a linear association between RTV and ADHD symptoms (Kuntsi and Klein, 2012). However, in conditions with fast event-rates and/or incentives, RTV normalises in individuals with ADHD (Johnson et al., 2009, Kuntsi and Klein, 2012). These findings suggest that optimal states of activation occur in conditions that elicit greater arousal via increased presentation speed, and/or greater effort via the prospect of reward. The CEM initially argued that an optimal state of arousal and activation could be induced based on event-rates that were neither too fast nor too slow (Sergeant, 2005), although the extent to which there is a single, optimal regulatory state has been difficult to prove (Johnson et al., 2009).

A third major theory concerns delay aversion. This is a motivational hypothesis specifying that individuals with ADHD experience a negative emotional reaction in response to delay. The initial supposition was of impulsive behaviour as a functional adaptation to avoid delay, reflecting a developmental consequence of children failing to engage with delay (Sonuga-Barke et al., 1992). The theory has been tested using a choice-delay paradigm, in which participants choose between a small-immediate or large-delayed reward. Preference for small-immediate rewards is considered an index of choice impulsivity, which is not however specific to the theory of delay aversion (Johnson et al., 2009). Some research has found greater choice impulsivity in children with ADHD in conditions in which impulsive responding reduces delay, supporting the delay aversion hypothesis (Sonuga-Barke et al., 1992, Dalen et al., 2004). However, other studies indicate that choice impulsivity is linked to the immediacy of rewards rather than the overall duration of delay (Marco et al., 2009, Scheres et al., 2006). This is in contrast to the original delay aversion hypothesis, which specified that impulsive responding should occur only when it leads to a shorter delay, as opposed to linking ADHD to reward processing. Revisions to the delay aversion theory therefore predict an interaction effect, in which the desire to escape delay compounds choice impulsivity in ADHD, as indicated by a general preference for small-immediate rewards that is strongest when it also reduces overall delay (Marco et al., 2009).

The neurobiological correlates of delay aversion and choice impulsivity were outlined in an influential dual pathway model of ADHD. The dual pathway model sought to reconcile conflicting theories of executive function deficits and delay aversion, arguing that the two represent distinct, heterogeneous pathways to ADHD-related behaviours from conceptually-related brain circuitry (Sonuga-Barke, 2002, Sonuga-Barke, 2003, Sonuga-Barke, 2005). This is based on empirical evidence of unique, uncorrelated associations of executive functions and delay aversion with ADHD (Dalen et al., 2004, Solanto et al., 2001, Sonuga-Barke et al., 2003). The dual pathway model hypothesised that executive functions are linked to the brain regions outlined above, primarily indexing cortical brain activity but with secondary links to subcortical regions. Delay aversion, including choice impulsivity, is presumed linked to fronto-striatal reward circuitry, including the orbitofrontal cortex, anterior cingulate, ventral

striatum and thalamus, thus implicating cortical and subcortical brain activity. Delay aversion theory has been criticised for being highly theoretical and appearing difficult to falsify, despite the potentially conflicting evidence regarding the role of choice impulsivity (Johnson et al., 2009).

A fourth major theory implicates developmental-dynamic processes in ADHD (Sagvolden et al., 2005). This neurotransmitter-based theory speculates that hypofunction of the mesolimbic dopamine branch results in a failure to modulate non-dopaminergic activity, leading in turn to two main alterations in behaviour. The first behavioural alteration is in the reinforcement of novel behaviours. Reinforcement is less effective with longer delays between a stimulus and reinforcer, and it is proposed that the time-limited window for reinforcement is shorter in individuals with ADHD than controls. This results in desirable behaviours being poorly reinforced, leading to the manifestation of inattention and motor impulsiveness symptoms of ADHD. The second behavioural alteration is the deficient extinction of existing behaviours. Extinction occurs when reinforcement stops, leading to cessation of the response behaviour. In individuals with ADHD poor extinction is thought to lead to excessive behaviours and behavioural variability – the respective symptoms of hyperactivity and cognitive impulsiveness. Thus, behavioural alterations could account for much of the socially inappropriate behaviour seen in ADHD.

The developmental-dynamic theory is seen as a comprehensive account of ADHD that attempts to explain all core symptoms of the disorder (Johnson et al., 2009). Indeed, the theory supposes that executive dysfunction and difficulties with state regulation can be accounted for by fundamental problems with behavioural acquisition, learning and retrieval (Sagvolden et al., 2005). Similarly, delay aversion is presumed to occur due to a shorter delay-of-reinforcement gradient. Although the primary dopamine deficiency is argued to occur in the mesolimbic system, dysfunction of mesocortical and nigrostriatal dopamine branches are also hypothesised to account for the respective symptoms of inattention and hyperactivity-impulsivity (Sagvolden et al., 2005). However, a potential limitation of this model is that it attempts to provide a homogeneous account of a heterogeneous disorder (Johnson et al., 2009).

The four outlined theories are not the only neurocognitive accounts of ADHD but represent major working hypotheses regarding cognitive performance deficits. Although the theories outline different primary deficits, they should not necessarily be considered competing. This is apparent from the use of the CEM to account for both executive functioning and regulatory deficits; and the use of a dual process model to account for delay aversion and executive dysfunction; and from use of a developmental-dynamic theory to account for all cognitive and behavioural symptoms of ADHD. This suggests that a multimodal explanation may be required to understand ADHD, possibly as a result of heterogeneity in the presentation of neurocognitive deficits (Johnson et al., 2009).

One example of neurocognitive heterogeneity is in the ability of cognitive performance tests to meaningfully discriminate between individuals with and without ADHD in clinical practice. Nigg et al (2005) illustrated this point using executive functioning as an example, showing that DSM-IV defined cases of ADHD could not be adequately identified based on the results of executive function tests alone. Instead, only a distinct subgroup of individuals presented with specific deficits in executive functioning, indicating heterogeneity in the cognitive performance deficits linked to ADHD (Nigg et al., 2005). Similar findings have since revealed that different profiles of cognitive performance are associated with individual differences among those with ADHD and with regard to ADHD symptoms among the general population (Fair et al., 2012, Nikolas and Nigg, 2013). One of these studies additionally found that combined and predominantly-inattentive subtypes of ADHD differed in the severity of cognitive performance deficits, with greater deficits in combined-type ADHD (Nikolas and Nigg, 2013). This further highlights the heterogeneous nature of ADHD, indicating potential subtype differences in cognitive performance deficits.

Another example of heterogeneity is seen in developmental studies of ADHD. Halperin and colleagues (2008) compared the neuropsychological profiles of ADHD persisters and remitters using a longitudinal design. Diagnostic status was determined at two time points: once in childhood at ages 7-11 years and once in adulthood at ages 17-21 years. Cognitive performance was assessed at both time points. At the first time point, individuals with ADHD performed

significantly worse than controls across a range of cognitive performance measures, including tests of vigilance, response inhibition, working memory, RTV and perceptual sensitivity. At the second time point, both persistent and remittent ADHD groups were found to differ significantly from controls on measures of RTV and perceptual sensitivity, suggesting that poor state regulation is a central, stable deficit in ADHD. In contrast, only ADHD persisters remained significantly different from controls on measures of executive functioning. This suggests that recovery from ADHD is associated with improvements in effortful control (Halperin et al., 2008). These results identify a developmental mechanism through which heterogeneity in cognitive performance may lead to individual differences in ADHD-related behaviours. Although this hypothesis is highly attractive, a recent systematic review concluded that persistent ADHD is characterised by poor performance across a range of cognitive tasks, as opposed to specific cognitive profiles, arguing that severity of cognitive performance deficits is the best determinant of developmental outcomes in ADHD (van Lieshout et al., 2013).

1.6.2 Endophenotypes

The term “endophenotype” was coined by two insect biologists to describe the geographical distribution of grasshoppers as a function of microscopic, internal features not readily apparent from the insect’s external phenotype (John and Lewis, 1966). Shortly thereafter, the term was applied to the study of schizophrenia to describe internal elements of the psychiatric phenotype that could be discovered via microscopic examination (Gottesman & Shields, 1967). Since then, the term has been used within the field of psychiatric genetics to refer to a range of intermediate phenotypes assumed to sit on the pathway between genes and behaviour (Gottesman and Gould, 2003).

The endophenotype hypothesis specifies that intermediate phenotypes can be used to reduce heterogeneity in psychiatric research, decreasing the number of physiological steps between genes and behaviour (Gottesman and Gould, 2003). Theoretically, this should assist with the detection of genes associated with disease. While the original hypothesis was that endophenotypes would be monogenic in origin, it is likely that endophenotypes are themselves complex,

with a polygenic basis much like behavioural psychiatric phenotypes (Gottesman and Gould, 2003). However, unlike most psychiatric phenotypes, endophenotypes should be more objective in terms of definition and measurement. Putative endophenotypes therefore include biochemical, endocrinological, neuroanatomical, neurophysiological and cognitive measures.

Table 1.2 Three recent definitions of *endophenotype*

	Gottesman and Gould (2003)
1	The endophenotype is associated with illness in the population.
2	The endophenotype is heritable.
3	The endophenotype is primarily state independent (manifests in an individual whether or not illness is active).
4	Within families, endophenotype and illness co-segregate.
5	The endophenotype found in affected family members is found in non-affected family members at a higher rate than in the general population.
	Preston and Weinberger (2005)
	"An intermediate phenotype (… endophenotype) is a quantitative biological trait that is reliable and reasonably heritable, i.e., shows greater prevalence in unaffected relatives of patients than in the general population. If a candidate intermediate phenotype is to provide meaningful information about a disorder, it should be associated with variant alleles that distinguish patients and their unaffected siblings from healthy controls on quantitative measures... The intensive search for such candidates is based in part on (the) ... assumption that intermediate phenotypes in schizophrenia (reflect) ... a less complex genetic architecture than the disorder as a whole."
	Canon and Keller (2006)
1	Endophenotypes should be heritable.
2	Endophenotypes should be associated with causes rather than effects of disorders.
3	Numerous endophenotypes should affect a given complex disorder.
4	Endophenotypes should vary continuously in the general population.
5	Endophenotypes should optimally be measured across several levels of analysis.
6	Endophenotypes that affect multiple disorders should be found for genetically related disorders.

Note: Table replicated from Kendler and Neale (2010).

The criteria for identifying putative endophenotypes were originally set out by Gottesman and Gould (2003) and have since been redefined by other authors (Cannon and Keller, 2006, Preston and Weinberger, 2005). The criteria across studies were recently summarised by Kendler and Neale (2010), replicated here in Table 1.2. Chief among these criteria are that putative endophenotypes be

associated with the psychiatric phenotype of interest in the general population, that they are heritable, and that they co-segregate with the phenotype of interest in the families of probands. Endophenotypes should therefore manifest as heritable, quantitative traits, meaning that family, twin and molecular genetic studies are appropriate for identifying and validating putative endophenotypes.

Kendler and Neale (2010) note that an inherent assumption of the endophenotype hypothesis is of a mediated relationship between genes and behaviour, in which genetic influences operate indirectly and via the putative endophenotype. For example, the genes associated with ADHD may have a direct effect on cognitive performance, which in turn directly influences behaviour. This is a causal statement that is rarely tested in empirical research, yet the same pattern of results could be accounted for by genetic pleiotropy. Pleiotropy occurs when the same sets of genes influence different traits but does not specify that one trait has a causal influence on another. It is important for research to test the extent to which an endophenotype causes a psychiatric phenotype versus the extent to which pleiotropy occurs, since this has implications for subsequent neurobiological and genetic research and the development of targeted treatments. Other salient issues include the need to test whether the same endophenotypes index multiple behavioural phenotypes and whether endophenotypes confer environmental as well as genetic risk. These conceptual issues can be addressed via experimental, longitudinal, familial and twin research (Kendler and Neale, 2010, Kendler et al., 1993a).

Because of the assumed cognitive basis of ADHD, one hypothesis is that measures of cognitive performance can be used as endophenotypes to assist in the discovery of genetic variants. This theory gains currency from the fact that cognitive performance is assumed to index brain structure and function, while remaining cost-effective and relatively easy to assess (Doyle et al., 2005b). Moreover the fact that multiple cognitive deficits are linked to ADHD suggests that cognitive performance across different domains could be used to identify aetiologically homogeneous groups of people with ADHD. The cognitive performance measures considered as putative endophenotypes are the same ones implicated in major neurocognitive theories of ADHD, including measures of response inhibition, working memory, delay aversion, choice impulsivity and

reaction time (Castellanos and Tannock, 2002, Doyle et al., 2005b). These processes could mediate the association between genes and ADHD, or may at least assist in the genetic mapping of ADHD risk genes. The extent to which cognitive performance variables are heritable and associated with ADHD, thus meeting the endophenotype criteria, has been considered in family, twin and molecular genetic research.

1.6.3 Family studies of cognitive performance and ADHD

Family studies have sought to establish the extent to which cognitive performance deficits and ADHD run in families. Much of the familial research on this topic has been conducted using data from the International Multi-centre ADHD Genetics project (IMAGE; Kuntsi et al., 2007); a sample of ADHD-affected probands and their siblings aged 5-18 years. Cognitive data were collected for a subset of the IMAGE sample, in addition to non-ADHD controls, to enable familial analyses of cognitive performance. The IMAGE findings are considered in detail here due to their relevance to analyses conducted in this thesis (chapters 6 and 7).

Familial research conducted using the IMAGE sample has examined cognitive performance measures including response inhibition (commission errors), vigilance (omission errors), working memory (digit span backwards) delay aversion, choice impulsivity, mean reaction time (MRT), RTV and IQ. Some studies utilised a familial modelling approach, whereas others have examined mean differences between ADHD probands, their unaffected siblings and controls. Findings on this topic are considered in chronological order.

The first study published using the IMAGE cognitive data examined reaction times (Andreou et al., 2007). MRT and RTV were measured using a reaction time task called the Fast Task (see section 2.2.4 for details on this measure). In the Fast Task baseline (slow event rate) condition, ADHD probands responded to stimuli with significantly slower MRTs and significantly greater RTV when compared to controls. The unaffected siblings of probands did not differ significantly from probands or controls for MRT, but showed significantly less RTV than probands. Familial analysis of MRT and RTV in the baseline condition

revealed modest phenotypic correlations with ADHD (r = 0.33 and 0.40, respectively). Using a bivariate application of the DF extremes approach, the authors estimated that familial effects accounted for 72% of the phenotypic correlation of ADHD with MRT and 63% of the correlation with RTV.

Rommelse et al. (2008) analysed cognitive performance and ADHD in the Dutch IMAGE cohort, examining the familial basis of inhibition, visuospatial and verbal working memory, and IQ. Linear mixed models indicated that ADHD probands and their ADHD-affected siblings performed significantly worse than controls across all cognitive performance measures, while the unaffected siblings of probands also performed significantly worse than controls across most tasks. This is indicative of familial associations between ADHD and executive dysfunction and between ADHD and IQ, although the latter finding was limited to verbal rather than performance IQ. Secondary analyses indicated that executive functions and IQ could be separated into independent factors that showed specific patterns of familial segregation (Rommelse et al., 2008).

Marco et al. (2009) examined choice impulsivity and delay aversion in ADHD probands and their siblings. The results of phenotypic analyses (also cited above, section 1.5.1) revealed a primary association of ADHD with choice impulsivity. ADHD probands, unaffected siblings and controls were classified as choice-impulsive or non choice-impulsive based on the number of times they selected smaller-immediate rewards. The siblings of choice-impulsive ADHD probands were significantly more likely to be choice-impulsive themselves, presenting with similar levels of choice impulsivity as found in the proband group. The siblings of non choice-impulsive ADHD probands were less choice impulsive and not significantly different from controls. This pattern of results suggests a familial association between choice impulsivity and ADHD.

Uebel et al. (2010) examined reaction times (MRT, RTV) and executive functioning (commission errors, omission errors) using the Go/No-go task (a reaction time task, see section 2.2.4). Consistent with results obtained for the Fast Task, MRT and RTV in the Go/No-go slow event-rate condition were significantly worse among ADHD probands. Trend analysis indicated that RTV was also somewhat impaired in unaffected siblings relative to controls,

suggesting a familial basis for RTV. A similar pattern of results was obtained for commission and omission errors, suggesting that familial effects drive response inhibition and vigilance (sustained attention). ADHD probands showed significant improvements in reaction time and executive functioning in the incentive condition of the Go/No-go task, with small improvements found among their unaffected siblings. This suggests that there may also be a familial basis to reward sensitivity in ADHD (Uebel et al., 2010b).

Kuntsi et al. (2010) conducted multivariate structural equation modelling to decompose covariation between ADHD and cognitive performance into familial and non-shared environmental components. Bivariate analyses indicated modest phenotypic correlations (r) and moderate to strong familial correlations (r_F) of ADHD with RTV (r = 0.39, r_F = 0.74), MRT (r = 0.36, r_F = 0.61), omission errors (r = 0.22, r_F = 0.48) and commission errors (r = 0.19, r_F = 0.45), and a weaker association between ADHD and choice impulsivity (r = -0.10, r_F = -0.39). Two common familial factors accounted for most of the familial variance (97.5%) in ADHD: the first factor accounted for 98% of the familial variance in MRT, 100% in RTV, and 85% of the total familial variance in ADHD; the second factor accounted for 82% of the familial variance in omission errors, 62% in commission errors, and 12.5% of the total familial variance in ADHD. Choice impulsivity did not correlate strongly with either factor (Kuntsi et al., 2010).

Wood et al. (2011) examined the role of IQ in relation to cognitive performance and ADHD. Previous studies had controlled for IQ prior to phenotypic and familial analyses, however this study explicitly tested whether the familial influences on ADHD and IQ were the same as the familial influences across ADHD and cognitive performance. The familial association between ADHD and IQ was largely independent of the familial associations between ADHD and cognitive performance: the percentage of familial covariation between cognitive performance and ADHD that was independent of IQ was 58% for MRT, 62% for RTV, 67% for commission errors, 52% for omission errors, and 53% for choice impulsivity. These findings indicate that cognitive performance deficits in ADHD are not due to a familial effect of low IQ (Wood et al., 2011b).

Frazier-Wood et al. (2012) examined the familial basis of cognitive performance among a Dutch subset of the IMAGE sample. Cognitive performance measures included a composite of intra-individual variability (primarily based on RTV across a series of tasks), MRT, digit span backwards (working memory), stop signal reaction time (response inhibition) and IQ. Familial modelling identified a two-factor model that accounted for 65% of the familial variance in ADHD, similar to that reported by Kuntsi et al. (2010). The first factor accounted for 100% of the familial variance in intra-individual variability, 60% in MRT, 12% in response inhibition, and 50% of the total familial variance in ADHD; the second factor accounted for 100% of the familial variance in working memory, 20% in response inhibition, 33% in IQ, and 15% of the total familial variance in ADHD.

There are several consistent patterns of results reported across the familial studies of cognitive performance in IMAGE. First, they identify a familial basis to the cognitive performance deficits implicated in the major neurocognitive theories of ADHD, suggesting that cognitive performance deficits run in families. Second, they identify familial co-segregation with ADHD, suggesting that cognitive performance deficits are viable candidate endophenotypes. Third, they indicate a separation of the different cognitive factors linked to ADHD, notably that measures of state regulation (i.e. MRT, RTV) can to a large extent be distinguished from measures of executive functioning (i.e. response inhibition, vigilance, working memory). This supports the notion of neuropsychological heterogeneity in ADHD and suggests that at least two neurocognitive factors share familial variance with ADHD. These findings are broadly consistent with those of independent studies using the same and different cognitive variables (Bidwell et al., 2007, Bitsakou et al., 2009, Doyle et al., 2005a, Gau and Shang, 2010, Loo et al., 2008, Nigg et al., 2004a, Schachar et al., 2005, Sonuga-Barke et al., 2010), indicating that results are not specific to a single study population or set of measures.

1.6.4 Twin studies of cognitive performance and ADHD

Twin research has further examined the extent to which cognitive performance deficits fulfill endophenotype criteria, testing whether cognitive performance traits are heritable, whether they covary with ADHD symptoms among the

general population, and whether genetic or environmental influences across measures of cognitive performance and ADHD are shared. Much of the twin research on this topic has been conducted using a single sample from the Study of Activity and Impulsivity Levels in children (SAIL; Kuntsi et al., 2006). As with the IMAGE sample, results from SAIL are highly relevant to research conducted in this thesis (chapters 6 and 7); hence the results of prior studies are considered here. Many of the measures used to assess cognitive performance in SAIL are the same as those used in IMAGE, allowing complementary research questions to be addressed.

Univariate twin analyses in SAIL have revealed moderate genetic influences for most measures of cognitive performance (Kuntsi et al., 2006). For the Go-No/go task heritability estimates across slow, fast and incentive conditions were 18-38% for commission errors, 31-54% for MRT and 10-43% for RTV; omission errors were rare and were therefore not examined. For the fast task, respective heritability estimates under baseline and fast incentive conditions were 55% and 23% for MRT, and 37% and 17% for RTV. Heritability estimates for delay aversion, measured using the Maudsley Index of Delay Aversion (for details on this measure, see section 2.2.4), were 18% under the no post-reward delay condition and 11% under a post-reward delay condition; however parameter estimates were distorted due to a ceiling effects under both conditions and should be interpreted with caution. For reverse digit span, heritability was estimated at 36%. Unlike the results for behavioural studies of ADHD symptoms, the heritability estimated for cognitive performance in SAIL was additive genetic in origin with no evidence of non-additive genetic effects. There were low to modest effects of the shared environment across the respective cognitive performance measures (0-27%), however all shared environmental parameter estimates were non-significant. These findings are broadly in line with those obtained in other studies (Ando et al., 2001, Kuntsi and Stevenson, 2001, Luciano et al., 2001, Rijsdijk et al., 1998, Vinkhuyzen et al., 2010).

The univariate analyses in SAIL indicated lower heritabilities for cognitive performance measures than are found for parent and teacher ratings of ADHD symptoms; however the use of composite indices of reaction time (data from the baseline condition of the Fast task and slow condition of the Go/No-go task)

somewhat increased the estimates of heritability to 60% for MRT and 48% for RTV. Even higher heritability estimates were obtained when measures were corrected to reduce measurement error, as indicated by test-retest reliabilities previously obtained for a subset of the SAIL sample (Kuntsi et al., 2005a). For the Go-No/go task, revised heritability estimates were 32-67% for commission errors, 49-83% for MRT and 53-100% for RTV. For the Fast task, revised heritability estimates were 29-73% for MRT and 26-70% for RTV. The heritability of composite reaction time measures increased to 73% for MRT and 68% for RTV. The high heritability found when correcting for measurement error is consistent with recent research by Young et al. (2009), in which genetic influences for a latent variable indexing cognitive inhibitory control were estimated at 100%. These findings indicate that the true extent of genetic influences on cognitive performance may be underestimated in twin studies when measurement error is not accounted for.

The first multivariate study to explore cognitive performance in SAIL took a phenotypic approach, examining the association of RTV, MRT and commission errors with ADHD (Kuntsi et al., 2009). This study initially compared the 5% of children scoring highest for parent and teacher-rated ADHD symptoms (n = 58) to the remainder of the SAIL sample (n = 1,098). On the Fast task, the high-ADHD group had slower MRTs and greater RTV in the baseline but not fast-incentive condition and showed significantly greater improvement across conditions when compared to the remainder of the sample. On the Go/No-go task, the high-ADHD group had significantly slower MRTs in slow and fast conditions, significantly greater RTV in slow, fast and incentive conditions, and committed more commission errors in slow and incentive conditions. Composite measures of MRT and RTV, generated by combining Fast task with Go/No-go data for both baseline and fast conditions, was similarly impaired in the high-ADHD group relative to the remainder of the sample. Continuous analyses were then conducted, in which ADHD symptom scores were correlated with cognitive performance using the entire SAIL sample, with partial correlation coefficients (controlling for age and sex) between $r = 0$ and $r = 0.26$. The strongest correlation was between ADHD and composite RTV assessed using data from the baseline conditions of the Fast task and Go/No-go task.

A second phenotypic study examined choice impulsivity and delay aversion, measured using the Maudsley Index of Delay Aversion in relation to the separate ADHD dimensions of hyperactivity-impulsivity and inattention (Paloyelis et al., 2009). Results revealed a significant association of choice impulsivity with inattentive ADHD symptoms but not hyperactivity-impulsivity. Additional analyses identified sex-specific effects, including an association of delay aversion with inattention in boys, and of hyperactivity-impulsivity with choice impulsivity in the no post-reward delay condition in girls. Categorical analyses also revealed that boys with extreme inattention scored significantly higher for choice impulsivity in the no post-reward delay condition.

Subsequent multivariate studies in SAIL have examined the genetic and environmental associations between cognitive performance and ADHD symptoms. Wood et al. (2010) examined the association of total ADHD symptoms with IQ, MRT and RTV. To reduce measurement error latent factors were created for MRT and RTV using data from the Fast task and Go/No-go baseline conditions. Two genetic factors showed significant associations with ADHD: the first genetic factor loaded significantly onto IQ and ADHD; the second genetic factor loaded onto MRT, RTV and ADHD. The same pattern of results was found when examining the loading of non-shared environmental factors onto IQ, cognitive performance and ADHD. In this study 92% of the covariation between ADHD and reaction time was independent of IQ, indicating differential aetiological associations between ADHD and IQ, and between ADHD and reaction time. The phenotypic correlation between MRT and RTV was particularly high (r = 0.97) while the genetic correlation was 1.00. This suggests that latent measures of MRT and RTV indexed alternate manifestations of the same underlying liability (Wood et al., 2010a).

One recent SAIL study has explored the genetic architecture of RTV further (Kuntsi et al., 2012), finding that RTVs in baseline conditions of the Fast task and Go/No-go task were highly correlated with RTV difference scores for the same tasks at both the phenotypic (r = 0.72 to 0.82) and genetic (r_G = 0.81 to 0.98) levels. Difference scores index the change in RTV between slow and fast/incentive conditions, indicating that the potential for change in RTV has the same aetiology as levels of baseline RTV. Parallel familial analyses were

conducted using the IMAGE sample, indicating a highly similar set of results (r = 0.83 to 0.90, r_F = 0.78 to 0.93).

Most recently, analyses in SAIL have revealed different genetic associations of the two ADHD symptom dimensions with RTV (r_G = 0.31 vs. 0.64), MRT (r_G = 0.19 vs. 0.56) and commission errors (r_G = 0.17 vs. 0.11) for hyperactivity-impulsivity and inattention respectively. Due to wide confidence intervals, the correlations of cognitive performance with hyperactivity-impulsivity versus inattention were not significantly different from one another; nonetheless, this pattern of results points towards subtype specific associations of cognitive performance with ADHD (Kuntsi et al., in 2013).

The multivariate results from within SAIL indicate that a number of cognitive performance variables are phenotypically associated with ADHD symptoms within a general population sample, consistent with the criteria for endophenotypes. The endophenotypic basis of cognitive performance is further established based on evidence of genetic associations with ADHD symptoms, where the strongest results to date have been obtained for measures of reaction time (MRT, RTV). These sets of results are consistent with those obtained from familial analyses within IMAGE, suggesting that the aetiological associations between cognitive performance and ADHD symptoms are similar in clinical and community-based samples. However, a key difference is that the twin analyses within SAIL have examined the two dimensions of ADHD separately, finding that measures of reaction time are more strongly associated with inattentive than hyperactive-impulsive behaviours.

1.6.5 Molecular genetic studies of cognitive performance and ADHD

Molecular genetic studies provide further evidence of genetic associations between cognitive performance deficits and ADHD, although results from studies to date are mixed. A systematic review of the literature, published in 2009, identified 29 studies that examined 10 candidate genes in relation to cognitive performance traits (Kebir et al., 2009). The most consistently studied genes were DRD4 and DAT1, both of which were significantly associated with ADHD affection status based on meta-analysis (Gizer et al., 2009; see section

1.5.2). For DRD4, there were consistent associations between the 7-repeat allele and *better* attention; absence of the 7-repeat was consistently associated with better vigilance, shifting and maintenance of attention, and lower RTV. This suggests that the association between DRD4 and ADHD affection status is unlikely to operate via cognitive performance deficits in these domains. Results across studies were mixed with regard to response inhibition, suggesting no effect of DRD4 on inhibitory processes. For DAT1, conflicting results were found regarding the association of the 10-repeat allele with response inhibition and vigilance, although the marker was consistently associated with greater RTV. This suggests that 10-repeat homozygotes may have particularly poor state regulation and indicates that DAT1 may be a suitable candidate gene for future research into ADHD. Although findings were also reviewed for eight other candidate genes (COMT, DBH, MAOA, DRD5, ADRA2A, GRIN2A, TPH2 and BDNF), the results across studies were inconsistent and often derived from underpowered samples, meaning that no firm conclusions regarding the association of these genes with cognitive performance deficits can be drawn.

Subsequent candidate gene analyses have further explored the genetic basis of cognitive performance deficits in ADHD. One recent study revealed a double dissociation of DRD4 and DAT1 with cognitive performance (Gizer and Waldman, 2012). In this study, the DRD4 7-repeat was significantly associated with deficient vigilance but not response inhibition, whereas the DAT1 10-repeat was significantly associated with deficient response inhibition but not vigilance. Cognitive performance deficits partially mediated the associations of these candidate genes with the respective ADHD symptoms of inattention and hyperactivity-impulsivity, suggesting that cognitive measures of inhibition and attention may represent genetically homogeneous endophenotypes for the two dimensions of ADHD. Another study found that the DRD4 7-repeat was associated with impaired cognitive performance across and range of tasks and specific to measures of working memory, visuospatial sequencing and shifting attentional set, but only in older children who were unaffected for ADHD.

Other recent studies have focused on choice impulsivity and delay aversion. The results of one study indicated a significant association of delay aversion but not choice impulsivity with the short allele of 5HTT in individuals with ADHD,

while neither trait was associated with DAT1. This suggests that choice impulsivity and delay aversion represent separable deficits that could be used to index genetically distinct subtypes of ADHD (Sonuga-Barke et al., 2011). Another, related study examined DAT1 and COMT associations with delay discounting, a measure of the subjective value of reinforcers over time that assesses choice impulsivity (Paloyelis et al., 2010a). Results indicated significantly greater impulsivity in a hypothetical delay-discounting task in carriers of the DAT1 10-6 repeat haplotype. This was a dosage effect, such that 10-6 heterozygotes performed significantly worse than homozygotes and non-carriers. There was also a significant association of the COMT Val-Met genotype with impulsive responding in the delay-discounting task, irrespective of ADHD diagnostic status. These findings suggest that specific genes may be associated with delay discounting in individuals with versus without ADHD.

Recent research has also used the linkage design to investigate the genetic basis of intra-individual variability in children and adolescents with ADHD and their unaffected siblings (Frazier-Wood et al., 2012). A composite measure of intra-individual variability was computed, primarily based on RTV across four different tasks. Results indicated suggestively significant linkage of composite intra-individual variability with three candidate regions on chromosomes 12, 13 and 17 (12q24.3, 13q22.2, 17p13.3), of which one region (17p13.3) was suggestively linked with ADHD in genome-wide linkage meta-analysis (Zhou et al., 2008). This suggests that variants within these regions may confer risk for an ADHD subtype characterised by poor state regulation.

Due to the small samples currently available there are as yet no published genome-wide studies of cognitive performance deficits in ADHD populations, although plans are in place to conduct genome-wide association analyses of cognitive performance data from international ADHD consortia in the near future (Asherson, 2013). Genome-wide analysis of executive functioning within the general population has thus far failed to identify any single gene effects at the adjusted GWAS significance threshold, likely due to the small effects of common alleles and insufficient sample sizes available (Cirulli et al., 2010). The available genetic data on cognitive performance should not be written off,

however, since it can be readily analysed using a polygenic approach as a further test the endophenotype hypothesis of ADHD.

1.7. TEMPERAMENT AND ADHD

1.7.1 A definition of personality and temperament

Personality refers to individual differences in thoughts, emotions and motivations, and has been widely studied by trait theorists seeking to characterise the facets of human behaviour (Bouchard Jr and Loehlin, 2001). At its broadest definition a personality trait is any continuously distributed psychological characteristic, although in most scientific research the term corresponds to an objectively defined characteristic in accordance with a specific theoretical framework or model (Bouchard Jr and Loehlin, 2001). Such frameworks typically assume a hierarchical structure to personality, with between three and nine overarching factors that account for clusters of personality traits (Bouchard and McGue, 2003, Verweij et al., 2012). The factors outlined across different personality models are moderately influenced by genes, with heritability estimates in the region of 30-60% (Bouchard Jr and Loehlin, 2001, Bouchard and McGue, 2003, Plomin et al., 2008). The remainder of phenotypic variation is almost exclusively accounted for via non-shared environmental influences, with little evidence of shared environmental effects.

Temperament refers to a relatively stable profile of early-emerging response patterns to external stimuli, reflected in individual differences in attentional, emotional and behavioural responses to the environment (Klein, 2011, Saudino, 2005). As with personality, it is assumed that temperament is biological in origin: most studies report heritability estimates in the region of 20-60%, with the remainder of phenotypic variance accounted for by the non-shared environment (Saudino, 2005). The constructs of temperament and personality are therefore similar (Bouchard Jr and Loehlin, 2001), to the extent that temperament can be viewed as a sub-domain of personality (Cloninger et al., 1993). However, a distinction is that temperament refers specifically to behaviours that emerge in early infancy and that endure over time, such as attention, activity level and emotionality (Cloninger et al., 1993; Saudino, 2005),

whereas personality is a broader construct that also incorporates specific cognitions, beliefs and values (Cloninger et al., 1993).

A number of different models of personality exist and although there are clear distinctions, there are also conceptual overlaps (see Bouchard Jr and Loehlin, 2001). For example, most models include a dimension of externalised, approach-related behaviours, referred to respectively as sensation seeking (Zuckerman and Cloninger, 1996), novelty seeking (Cloninger et al., 1993) and extraversion (Costa and McCrae, 1995, Eysenck and Eysenck, 1985). Similarly, most models characterise internalised, avoidant behaviours, referred to as neuroticism (Costa and McCrae, 1995, Eysenck and Eysenck, 1985, Zuckerman and Cloninger, 1996) and harm avoidance (Cloninger et al., 1993). The remainder of this thesis will focus primarily on a single model of personality, Cloninger's psychobiological model (Cloninger, 1994, Cloninger et al., 1993).

1.7.2. Cloninger's Psychobiological model

Cloninger's psychobiological model defines personality as *"an individual's psychophysical systems that determine [his or her] unique adjustment to [his or her] environment"* (Allport, 1937; as cited in Cloninger et al., 1993). One of the central tenets of this model is that personality is determined by both biological and social factors, which are divided into the separate domains of temperament and character. These domains are assessed using the Temperament and Character Inventory (TCI, Cloninger et al., 1993) and its subsequent revisions (Cloninger et al., 1994, Luby et al., 1999). The psychobiological model additionally assumes that the different dimensions of temperament and character are aetiologically independent, and that the interaction between temperament and character is responsible for psychopathology and wellbeing.

Cloninger's temperament domain refers to a set of automatic, biologically-based response patterns to external stimuli, thought to develop in early childhood and with an aetiology rooted in neurobiological and genetic factors. This definition of temperament is purportedly derived from genetic and biological studies in humans and animals, where there is evidence of heritable biases in learning, memory, processing and affect (Cloninger, 1987; see also Cloninger et al,

1993, for review). The psychobiological model outlines four homogeneous dimensions of temperament that correspond to such biases: novelty seeking (a heritable bias in the activation of behaviours); harm avoidance (a heritable bias in the inhibition or cessation of behaviours); reward dependence (a heritable bias in the maintenance or continuation of ongoing behaviours); and persistence (a heritable bias in pervasiveness, despite frustration and fatigue). Persistence was originally considered a sub-component of the reward dependence dimension (Cloninger, 1987), but emerged as a separate factor in subsequent research (Heath et al., 1994).

Cloninger's character domain refers to concepts of the self, or the *"who"*, *"what"* and *"why"* of an individual's existence (Cloninger et al., 1993). Character is seen as having social origins based on insight learning (i.e. verbal learning and the development of learning sets) and the reorganisation of self-concepts throughout the course of development. Character is therefore considered more malleable than is temperament, and with increasing age plays a role in moderating temperamental responses to the environment. Cloninger outlines three concept-based dimensions of character: self-directedness (individual differences in self-control and self-regulation); co-cooperativeness (individual differences in identification with and acceptance of others); and self-transcendence (individual differences in identification with essential and consequential parts of a unified whole). For the remainder of this thesis only Cloninger's temperament dimensions will be considered in detail due to their relevance to the research presented in chapter 4.

1.7.3 The aetiology of temperament

Empirical research has sought to validate the factor structure of temperament proposed in the psychobiological model, supported in some factor analyses (Brandstrom et al., 1998, Cloninger et al., 1993, Heath et al., 1994) but not others (Farmer and Goldberg, 2008, Garcia et al., 2012). A recent meta-analysis identified low-to-modest correlations between the four dimensions of temperament (Miettunen et al., 2008), generally supporting Cloninger's assertion of independence between dimensions. The strongest pairwise associations were between novelty seeking, harm avoidance and persistence

(correlations ranging from -0.27 to -0.14), with weaker pairwise associations with reward dependence (correlations ranging from 0.04 to 0.10). Research conducted by the same group has also demonstrated significantly higher reward dependence and harm avoidance scores in females than males via a separate meta-analysis (Miettunen et al., 2007); and has shown significant across-country differences in mean temperament scores, suggestive of cross-cultural variation (Miettunen et al., 2006).

Twin studies indicate that Cloninger's temperament dimensions are moderately heritable throughout adolescence and adulthood, with heritability estimates of 18-46% for novelty seeking, 36-49% for harm avoidance, 35-44% for reward dependence, and 0-37% for persistence (Ando et al., 2002, Ando et al., 2004, Gillespie et al., 2003, Heath et al., 1994, Heiman et al., 2003, Heiman et al., 2004, Stallings et al., 1996). Shared environmental effects appear negligible, while approximately half the phenotypic variance is explained by the non-shared environment. An exception to the pattern of results was reported in a twin study of childhood temperament, in which novelty seeking was explained by shared and non-shared environmental influences, and reward dependence and persistence were entirely accounted for by non-shared environmental effects (Isen et al., 2009). However, a high degree of measurement invariance and error across the self-reported temperament dimensions in this study could account for these anomalous results. Aetiological sex differences have been found in only a few studies of the temperament dimensions to date (Keller et al., 2005, Stallings et al., 1996).

The results of twin studies consistently suggest that the heritability of temperament is additive genetic in origin, finding either no evidence to suggest genetic non-additivity or having dropped non-significant non-additive genetic parameters from models. However, a twin-plus-sibling study revealed that non-additive genetic influences accounted for 11-35% of the total variance in the four temperament dimensions (Keller et al., 2005). These results are compelling, since twin-sibling models have greater power to detect non-additive genetic effects, suggesting that studies using the classical twin design may over-estimate the additive genetic influences on temperament. An even more

powerful approach is the extended twin-family design (Keller et al., 2010), although this has yet to be applied to study Cloninger's psychobiological model.

Multivariate twin studies have revealed moderate additive genetic and non-shared environmental correlations between the different dimensions of temperament (r_A = -0.49 to 0.42, r_E = -0.51 to 0.21) (Ando et al., 2002; Gillespie et al, 2003). These results are generally in line with the temperament factor structure proposed by Cloninger, despite indicating that the different dimensions are not entirely aetiologically independent. Non-independence was further found in a factor analysis of the genetic and environmental correlations between temperament and character, which showed that four genetic factors and three non-shared environmental factors were sufficient to capture all aetiological influences across the seven temperament and character dimensions (Ando et al., 2004). There are as yet no longitudinal twin studies of the psychobiological model; however cross-sectional analyses suggest only marginal effects of age on mean temperament scores (Heiman et al., 2003). The available evidence therefore supports the assertion that temperament is relatively stable and enduring over time.

Consistent with the heritability estimates derived from quantitative genetic studies, research has sought to demonstrate a molecular genetic basis for the dimensions of temperament, primarily genetic association studies.

Candidate gene studies have reported associations of several loci with Cloninger's temperament dimensions, including markers for dopaminergic, noradrenergic, serotonergic and GABA genes, and polymorphisms unrelated to neurotransmitter systems (Comings et al., 2000). Some studies have specifically tested the early hypothesis that the dimensions of temperament are differentially associated with neurotransmitter systems, with novelty seeking linked to the dopaminergic system, harm avoidance to the serotonergic system, and reward dependence to the noradrenergic system (Cloninger, 1987). Association of the dopamine D4 receptor gene (DRD4) with novelty seeking and of the serotonin transporter gene (5HTT) with harm avoidance was initially well documented (Savitz and Ramesar, 2004); however recent meta-analyses do not support such a monistic view. In one study the C521T variant of DRD4

explained as much as 3% of the variance in novelty seeking, while association with the variable number tandem repeat (VNTR) was non-significant (Munafo et al., 2008). In a second meta-analysis, the association of the serotonin transporter linked promotor region (5HTTLPR) with harm avoidance was found to be non-significant (Munafo et al., 2009). In general, these results indicate that temperament dimensions are likely polygenic in origin.

Two GWAS have examined Cloninger's dimensions of temperament; however neither study identified associations at the genome-wide level of significance (Service et al., 2012, Verweij et al., 2010). One potential explanation for the lack of significant results is that temperament traits arise primarily as a result of rare genetic mutations and genetic non-additivity, as opposed to the additive effects of common genetic variation (Verweij et al., 2010). This hypothesis was recently tested using the GCTA method (Verweij et al., 2012). Data from the two previous GWAS were pooled and the heritability of each temperament dimension was estimated as a function of the variance explained by all autosomal loci (269,616 SNPs, after quality control). SNP-wide heritability estimates were 9.9% for NS, 6.6% for HA, 4.2% for RD and 8.1% for PS. There was also evidence of inbreeding effects for the dimensions of novelty seeking, harm avoidance and reward dependence. These findings are consistent with a mutation-selection bias hypothesis, whereby additive genetic effects account for a relatively small proportion of the variance in temperament traits, but an accumulated mutation load, consisting of mildly deleterious rare alleles and/or genetic dominance and epistasis, accounts for much of the broad-sense heritability (Verweij et al., 2012). This echoes the finding of non-additive genetic influences in twin research (Keller et al., 2005).

1.7.4. Temperament and ADHD

There are conceptual arguments for examining the associations between Cloninger's temperament dimensions and ADHD. First, ADHD symptoms have been shown to manifest at a continuous level throughout the general population (see section 1.3.1), much like the dimensions of temperament. It is therefore plausible that there will be covariation between the symptoms of ADHD and dimensions of temperament. If so, ADHD could potentially be viewed as

extreme levels of continuously expressed temperamental traits. Second, research into personality more generally (i.e. not specific to the psychobiological model) suggests that different profiles of temperament can be used to characterise distinct profiles or subtypes of ADHD (Martel et al., 2011, Nigg et al., 2004b), thereby improving taxonomy of the disorder. Profiles of temperament and personality have similarly been used to characterise distinct patterns of psychiatric comorbidity in ADHD (Martel et al., 2010b). This suggests that specific profiles of temperament could be used to identify more homogeneous subtypes of ADHD. Third, because of its emergence in early infancy, temperament could be used prospectively to predict the development of ADHD (Nigg et al., 2004b, Taurines et al., 2010). However, since ADHD is also characterised by early developmental onset, this theory requires testing using longitudinal designs.

There is substantial evidence of phenotypic associations between ADHD and temperament across the lifespan. Clinical studies indicate that children and adults with ADHD score significantly higher than controls for the dimension of novelty seeking (Anckarsäter et al., 2006, Cho et al., 2008a, Cho et al., 2009, Downey et al., 1997, Faraone et al., 2009, Jacob et al., 2007, Lynn et al., 2005, Salgado et al., 2009, Sizoo et al., 2009, Smalley et al., 2009, Tillman et al., 2003, van Dijk et al., 2011). Most of these studies also indicate an association with increased harm avoidance, while evidence of associations with lower reward dependence and persistence have been reported less consistently (Cho et al., 2008a, Cho et al., 2009, Faraone et al., 2009, Tillman et al., 2003).

Some of these analyses examined the differential association of temperament with the two ADHD symptom domains. Lynn et al. (2005) found that novelty seeking was predictive of higher inattentive and hyperactive-impulsive symptom scores, while Faraone et al. (2009) found that inattentive and hyperactive-impulsive symptoms correlated positively with novelty seeking and harm avoidance, but negatively with reward dependence and persistence. These findings suggest that the ADHD symptom dimensions are characterised by similar profiles of temperament. In contrast, Salgado et al. (2009) found positive associations between inattention and harm avoidance; between hyperactivity-impulsivity, novelty seeking and persistence; and of combined-type ADHD with

novelty seeking. This indicates that there may be differential relationships of temperament with the two symptom dimensions of ADHD.

A limitation of the findings reviewed above is that they come from clinical studies, which may be subject to referral bias. However, a recent adult population study reported similar results, including a positive association of total ADHD symptoms with novelty seeking and harm avoidance, of inattentive symptoms with harm avoidance, and of hyperactive-impulsive symptoms with persistence (Gomez et al., 2012). Similarly, a community-based study of school children identified positive correlations of hyperactive-impulsive and inattentive symptoms with novelty seeking, but negative correlations with persistence (Yoo et al., 2006).

1.7.5. Twin studies of temperament and ADHD

Only three published twin studies have examined the aetiological relationship between Cloninger's temperament dimensions and ADHD. The first of these studies (Young et al., 2000) investigated the association between total ADHD symptom scores and novelty seeking via multivariate modelling that also included conduct problems and substance use. Covariance among the four phenotypes was best accounted for via a latent factor, termed *"behavioural disinhibition"*. Eighty-four percent of the variance in the latent factor was explained by additive genetic influences, and the latent factor accounted for 46% of the variance in ADHD symptoms and 22% of the variance in novelty seeking. A residual non-additive genetic component of variance loaded onto both ADHD and novelty seeking, and explained an additional 6% and 19% of their respective variances.

The second published study (Young et al., 2009b) was a follow-up to the first and used a partially overlapping sample to examine the construct of behavioural disinhibition at two time points (mean ages 12.4 and 17.4 years). At time 1, 59% of the variance in the latent factor was explained by additive genetic influences. This latent factor accounted for 72% of the variance in ADHD symptoms, but only 5% of the variance in novelty seeking. At time 2, 43% of the variance of the latent factor was explained by additive genetic influences, and the latent factor

accounted for 58% of the variance in ADHD symptoms and 13% of the variance in novelty seeking. A similar pattern of results emerged when ADHD symptoms of hyperactivity-impulsivity and inattention were modelled separately.

The third published study (Wood et al., 2011a) examined the association between symptoms of hyperactivity-impulsivity and novelty seeking using cross-sectional and longitudinal data. ADHD symptoms were assessed during childhood only, while novelty seeking was assessed during childhood and again in adolescence. Two multivariate models provided a similar fit to the data. The first was a correlated factors solution of the Cholesky decomposition, in which non-additive genetic influences were significantly correlated between hyperactivity-impulsivity and novelty seeking at the first time point (r_D = 0.81). The second model was a direction of causation model, in which variation in each phenotype was divided into a latent trait and residual variance. Bidirectional causal paths between at time 1 accounted for 10% of the variance in hyperactivity-impulsivity and 12% of the variance in novelty seeking, while a unidirectional causal path from hyperactivity-impulsivity at time 1 accounted for 6% of the variance in novelty seeking at time 2.

The results across twin studies consistently indicate a genetic association between ADHD symptoms and novelty seeking, however the extent to which ADHD symptoms are aetiologically related to Cloninger's other temperament dimensions has yet to be explored. Similarly, all published twin studies have examined ADHD and temperament in childhood, meaning that the associations in adulthood remain unknown.

1.7.6. Molecular genetic studies of temperament and ADHD

Molecular genetic studies of the relationship between Cloninger's temperament dimensions and ADHD have taken a candidate gene approach. A systematic search of the literature identifies 12 published studies (Cho et al., 2008b, Cho et al., 2008c, de Cerqueira et al., 2011, Frank et al., 2004, Grevet et al., 2007, Jacob et al., 2012, Lynn et al., 2005, Nyman et al., 2012, Nyman et al., 2007, Reif et al., 2011a, Schlaepfer et al., 2007, Weissflog et al., 2012) examining 13 genes (DRD1, DRD2, DRD3, DRD4, DRD5, 5HTT, ARDA2A, ARDA2C, NET1,

PRKCG, DIRAS2, PPP2R2C, KCNIP4; see http://www.ncbi.nlm.nih.gov/gene). However, these studies have either failed to identify significant associations of candidate markers with ADHD and temperament simultaneously, have reported results that were uncorrected for multiple testing, or have failed to identify significant results once corrections for multiple testing were made. Further work is therefore required to characterise the molecular genetic association between temperament and ADHD.

1.8 EMOTIONAL LABILITY

1.8.1 A definition of emotional lability

Emotional lability is a broad term used to refer to a set of symptoms including irritability, low frustration tolerance, temper outbursts, mood volatility and dysphoria. This set of behaviours has been increasingly linked to ADHD, in addition to other psychiatric phenotypes (Kring and Sloan, 2010), and there are now increased efforts to understand the aetiology of emotional problems in children, adolescents and adults with ADHD. Throughout this thesis, the term *emotional lability* will be treated as synonymous with other, related concepts outlined in scientific literature including *emotional dysregulation* (Reimherr et al., 2005b), *mood dysregulation* (Stringaris et al., 2012a), *emotional impulsiveness* (Barkley and Fischer, 2010), *mood instability* (Skirrow et al., 2009), *deficient emotional self-regulation* (Surman et al., 2011) and *mood lability* (American Psychiatric Association, 1994). It is recognised that although there are some conceptual differences between these definitions, the overarching set of symptoms they describe appears highly similar.

1.8.2. Emotional lability and ADHD

The renewed scientific interest in ADHD and emotional lability reflects longstanding evidence of a clinical association. Historic conceptualisations of ADHD included problems of emotional lability at their core, as reviewed by Barkley (2010) and summarised here. Early examples included the symptoms of emotional reactivity and anger reported to co-occur with attention problems by Alexander Crichton in 1798, and the co-occurrence of attention problems,

impulsiveness and poor emotional control documented by George Still in 1902. Later examples were identified in the criteria for minimal brain dysfunction (MBD), which included core symptoms of hyperactivity, impulsiveness, short attention span, perseveration, and emotional lability, and in the description of explosive behaviour and low frustration tolerance as symptoms of hyperkinetic impulse disorder. Interest in emotional lability continued to characterise research into hyperactive-impulsive behaviours up until the 1970s, when a paradigm shift occurred and the symptoms of hyperactivity-impulsivity and inattention were considered more relevant. This shift was reflected in the diagnostic criteria for ADHD from DSM-II onwards, which consistently recognised as core symptoms hyperactivity-impulsivity and inattention. This focus was retained in DSM-IV, in which symptoms of emotional lability were listed only as an associated feature of ADHD (American Psychiatric Association, 1994). The same description is now also retained in DSM-5 (American Psychiatric Association, 2013).

Despite the narrow diagnostic criteria for ADHD, recent research has continued to document an association between the symptoms of hyperactivity-impulsivity, inattention and emotional lability, to the extent that some researchers have argued that emotional lability should be seen as an integral, rather than associated, feature of ADHD (Barkley, 2010, Corbisiero et al., 2013, Retz et al., 2012, Skirrow et al., 2009). The main evidence for this comes from three converging lines of clinical research.

First, clinical studies have indicated that the core symptoms of ADHD co-occur with symptoms of hyperactivity-impulsivity and inattention. Research into children has revealed significantly higher levels of emotional lability in those with ADHD (Anastopoulos et al., 2011), while analyses of the two separate ADHD dimensions has revealed a significantly stronger association of emotional lability with hyperactive-impulsive than inattentive symptoms (Sobanski et al., 2010). Research has similarly identified significantly higher levels of emotional lability among adults with ADHD when compared to controls, finding that EL symptoms occur nearly as frequently in adults with ADHD as the symptoms of hyperactivity-impulsivity and inattention themselves (Barkley and Murphy, 2010). Other studies have reported greater emotional lability in adults

with persistent ADHD (Barkley and Fischer, 2010) and have identified robust case/control differences in emotional lability even after controlling for residual symptoms of psychiatric comorbidity (Skirrow and Asherson, 2013). These adult studies also indicate a stronger association of emotional lability with hyperactive-impulsive than inattentive ADHD symptoms.

Second, clinical studies have identified significant associations between emotional lability and functional impairments. In studies of adult ADHD this association has remained significant even after controlling for symptoms of hyperactivity-impulsivity and inattention (Barkley and Murphy, 2010, Barkley and Fischer, 2010, Skirrow and Asherson, 2013). The impairments linked to ADHD across these studies include difficulties in home functioning, social interactions, community activities, spousal/partner relationships, money management, driving offences, risk taking behaviour, and leisure/recreational activities. In children with ADHD there is evidence that symptoms of emotional lability partially mediate the association between ADHD and functional impairments, including problems with social skills and tasks of daily living (Anastopoulos et al., 2011). These studies have additionally linked ADHD and emotional lability to comorbid symptoms including anxiety, depression, and disruptive and antisocial behaviours.

Third, clinical studies into treatment effects have consistently identified a concomitant decline in symptoms of hyperactivity-impulsivity, inattention and emotional lability in response to stimulant and atomoxetine medication (Marchant et al., 2011a, Marchant et al., 2011b, Reimherr et al., 2005b, Reimherr et al., 2007, Rosler et al., 2010). This literature comes exclusively from adults and there are as yet no published studies into the effects of methylphenidate or atomoxetine on emotional lability in childhood. However, research has demonstrated concomitant medication effects on symptoms of ADHD and aggression in children and adolescents (Connor et al., 2002, Nevels et al., 2010), suggesting an effect on emotional-type symptoms. The effects of non-pharmacological interventions on emotional lability in ADHD have been less widely researched, although there is some evidence of a reduction in ADHD and emotional lability symptoms in children following parent training (Bor et al., 2002, Herbert et al., 2013). Manualised CBT, dialectical behaviour

therapy (DBT) and mindfulness-based programmes for adolescents and adults with ADHD typically include modules on emotion and self regulation (Philipsen et al., 2010, Young and Bramham, 2012, Young and Ross, 2007, Zylowska et al., 2008), although further research into their efficacy for the treatment of emotional lability is required.

Taken together, clinical studies have identified strong evidence of an association between ADHD and the symptoms of emotional lability across the lifespan, providing some support for the theory that emotional lability reflects a core component of ADHD (Barkley, 2010, Skirrow et al., 2009). A central tenet of this theory is that the symptoms of emotional lability in ADHD are chronic and persistent, as opposed to episodic, enabling them to be distinguished from the emotional symptoms of other disorders. This is reflected in studies that use experience sampling methods, in which children (Rosen and Epstein, 2010) and adults (Skirrow and Asherson, unpublished data) with ADHD present with chronic patterns of emotional lability over successive periods of time. Yet despite this, symptoms of chronic emotional lability are not considered specific to ADHD and are also characteristically seen in other psychiatric disorders, notably oppositional defiant disorder (ODD) and pediatric bipolar disorder. While these disorders are not the primary focus of this thesis, their overlap with symptoms of emotional lability and ADHD warrants some consideration.

ADHD and ODD are highly comorbid in childhood and adolescence (see section 1.3.5), perhaps because of an overlap in the symptoms of emotional lability (Barkley, 2010). Factor analyses have identified irritable, headstrong and hurtful components of ODD, of which the irritable dimension incorporates symptoms of chronic emotional lability (Ezpeleta et al., 2012, Kuny et al., 2013, Rowe et al., 2010, Stringaris and Goodman, 2009b). Irritable ODD in childhood is predictive of anxiety and depression in later life (Burke, 2012, Stringaris and Goodman, 2009a, Stringaris et al., 2012b), however there is mixed evidence as to whether ADHD is primarily related to the irritable (Ezpeleta et al., 2012, Kolko and Pardini, 2010) versus headstrong (Ezpeleta et al., 2012, Stringaris and Goodman, 2009a) components of ODD. One clinical study of children and adolescents with ADHD identified substantial associations between emotional lability, ODD and hyperactive-impulsive symptoms (Sobanski et al., 2010).

Because both ODD and ADHD are associated with emotional symptoms, research on this topic might improve understanding of the relationship between emotional lability and ADHD. For example, one recent twin study found that almost all genetic influences on ODD symptoms were shared with symptoms of hyperactivity-impulsivity (Wood et al., 2009b). Comorbid ADHD and ODD symptoms also simultaneously respond to medication (Biederman et al., 2007) and to parent training interventions (Thompson et al., 2009). These findings point towards a common aetiology for ODD and ADHD that could account for some of the overlap seen between ADHD and emotional lability; however the aetiological relationship between ADHD and *'pure'* symptoms of irritability/emotional lability remains unknown.

ADHD and bipolar disorder (BD) are highly comorbid in adulthood (see section 1.3.5), with a strong degree of familial association (Faraone et al., 2012). Both disorders share symptoms of distractibility, psychomotor agitation, excessive talkativeness and emotional lability, based on the primary and associated features outlined in DSM-IV. However key distinctions are that symptoms of emotional lability are episodic rather than chronic in BD and thus classified as mania, and that episodes of mania refer to extended periods of sustained abnormal mood states (see Skirrow et al., 2012).

More controversial is the association between ADHD and pediatric BD (PBD), which is first diagnosed in childhood and has been characterised by some authors as incorporating chronic rather than episodic symptoms of emotional lability. One set of criteria used to define PBD is the presence of severe attention problems, aggressive behaviours and anxious/depressed symptoms based on the Child Behaviour Checklist (CBCL) (Achenbach and Rescorla, 2003), defining a group with severe emotional dysregulation that represents around 1% of the general child population (Biederman et al., 2013b, Faraone et al., 2005a). Studies that have applied these criteria find extremely high rates of comorbidity between PBD and ADHD (Biederman et al., 2005a, Biederman et al., 2005c), although this is hardly surprising since items from the CBCL attention problems scale (including hyperactive-impulsive and inattentive behaviours) are essential features of both disorders.

More recent research has argued that PBD can be distinguished from ADHD with emotional lability (referred to as deficient emotional self-regulation, DESR) based on the severity of symptoms (Biederman et al., 2013a, Biederman et al., 2012a), although the extent to which DESR and PBD represent qualitatively distinct entities remains unclear. Nonetheless, PBD-type symptoms are now outlined as a distinct diagnostic entity in DSM-5, termed *disruptive mood dysregulation disorder* (American Psychiatric Association, 2013). These issues reflect a much wider debate within the scientific community regarding the validity of PBD and the demarcation of ADHD and BD across the lifespan, discussed in detail elsewhere (Leibenluft, 2011, Skirrow et al., 2012). Despite this polemic, one broad interpretation of the BD/PBD literature is that it provides further evidence of an association between emotional lability and ADHD.

1.8.3 Cognitive theories of emotional lability and ADHD

The shared treatment effects for symptoms of ADHD and emotional lability, in particular the co-action of medication, are suggestive of a common aetiology for these different behavioural dimensions. This could reflect shared neurobiological substrates and common pathways from genes to behaviour. In reviewing the literature, Skirrow et al. (2009) identified a range of neurobiological factors implicated in the development of ADHD and/or emotional lability symptoms, including abnormalities in amygdalo-prefrontal pathways such as the prefrontal cortex, basal ganglia, caudate, striatum, thalamus, hippocampus and cerebellum. Similar findings have also been described elsewhere (Hermann et al., 2010). Many of these are the same regions implicated in cognitive theories of ADHD (see section 1.6) and one way of testing for common neurobiological substrates for ADHD and emotional lability is to examine their associations with cognitive performance.

Skirrow et al. (2009) expand the existing literature on cognitive performance deficits in ADHD (section 1.6.1) to outline three cognitive hypotheses of emotional lability. The first hypothesis concerns executive functioning. It has previously been argued that executive functions represent a general construct responsible for the regulation of behaviours and emotions in accordance with

social norms (Jurado and Rosselli, 2007), therefore executive dysfunction in ADHD may lead to poor emotional regulation and ultimately emotional lability. The second hypothesis is that greater RTV in ADHD reflects a state regulation deficit, which may also account for variability in the regulation of other domains, including emotions. Barkley (2010) similarly argues the case for two, similar pathways to emotional lability symptoms in ADHD. The first reflects an inhibitory deficit, leading to impulsivity in emotions and hyperactive-impulsive behaviours, while the second reflects a self-regulatory deficit, leading to inattention and difficulties in generating countervailing emotional responses in line with social norms. The third hypothesis outlined by Skirrow et al. (2009) concerns delay aversion and draws heavily on work from Sonuga-Barke, arguing that aversion to delay leads to increased frustration, which is externalised as negative emotional reactions (Sonuga-Barke, 2002, Sonuga-Barke, 2003, Sonuga-Barke, 2005).

To date only two studies have directly examined the relationship between cognitive performance, ADHD and emotional lability. The first of these studies used the IMAGE sample and sought to directly test the hypotheses proposed by Skirrow et al. (2009). This study found that a modest amount of the variance in emotional lability could be explained by measures of executive functioning (inhibition = 19%; vigilance = 28%; working memory = 15%), MRT (36%), RTV (30%), choice impulsivity (11%) and immediate drive for reward (15%), in children and adolescents with ADHD (Banaschewski et al., 2012). There was no significant association with delay aversion. However, after controlling for symptoms of ADHD these associations were attenuated to a non-meaningful level. This change is consistent with a mediation effect (Baron and Kenny, 1986), whereby the association between cognitive performance and emotional lability is indirect and operates via the symptoms of ADHD. Because the strongest association prior to controlling for ADHD symptoms was with RTV, Banaschewski et al. argue that a state regulatory deficit best accounts for the association of ADHD with emotional lability based on available measures.

The second study found that adults with ADHD and DESR (i.e. severe symptoms of EL) did not differ significantly from adults with ADHD without DESR across measures of executive functioning including vigilance, planning

and set-shifting, interference control, visual scanning and verbal learning (Surman et al., 2013). This suggests that there is no specific profile of cognitive performance that differentiates individuals with and without emotional lability among an adult ADHD sample.

Overall, there is no indication that the cognitive deficits linked to ADHD lead directly to the symptoms of emotional lability; however further research on this topic is required, including replication among a general population sample. Both sets of findings are consistent with a hypothesis of mediation, in that there was no association between cognitive performance and emotional lability after controlling for the core symptoms of ADHD. Yet despite these results there could still be a common aetiology for the symptoms of ADHD, emotional lability and cognitive performance. This could be an indirect, mediated liability, or could reflect pleiotropic genetic effects that operate across behaviours. These questions can be addressed via quantitative and molecular genetic research.

1.8.4 Family studies of emotional lability and ADHD

Few studies have been conducted examining the familial basis of emotional lability and ADHD, and those that have reveal an inconsistent pattern of results. The largest study to date made use of the IMAGE sample to examine the associations between ADHD symptoms, emotional lability and other psychiatric comorbidities (Sobanski et al., 2010). ADHD probands were stratified into three groups based on levels of emotional lability. These groups differed significantly for ADHD symptoms scores, levels of ODD and conduct problems, peer problems, symptoms of anxiety and psychosomatic difficulties, with the most severe profile of symptoms found for the high emotional lability group. Familial analyses indicated higher levels of emotional lability in the siblings of probands who were high in emotional lability, suggesting a familial effect. However, emotional lability in the probands was not significantly associated with ADHD in the siblings, nor was there evidence of familial co-segregation. These results indicate that, although ADHD and emotional lability frequently co-occur, they do not do so due to familial effects.

Two other family studies have been published on the topic of DESR. The first of these studies examined DESR in adults, assessed using nine items from the Barkley Current Behaviour Scale (Surman et al., 2011). Results revealed significant familial co-segregation of ADHD and DESR symptoms, but with greater levels of ADHD plus DESR in the siblings of probands who also had ADHD and DESR. This was interpreted as evidence that ADHD with DESR represents a distinct familial subtype of ADHD. The second study examined DESR in children, assessed using the attention problems, anxious/depressed and aggressive scales of the CBCL (Biederman et al., 2012d). Analyses indicated a linear association between rates of DESR in siblings, such that they were lowest in the siblings of controls, higher in the siblings of ADHD probands, and higher still in the siblings of ADHD probands with DESR. This indicates a familial effect. Because DESR was measured using the CBCL, this study also attempted to examine the association between ADHD, DESR and PBD, but was unable to do so due to the small number of participants in the PBD group.

1.8.5 Twin studies of emotional lability and ADHD

To date, there are no published twin studies directly examining the aetiological relationship between the symptoms of emotional lability and ADHD. Nonetheless, twin studies of related phenotypes are potentially informative. First, univariate twin studies yield heritability estimates of 50-70% for symptoms pertaining to emotional lability (Boomsma et al., 2006, Hudziak et al., 2005, van Beijsterveldt et al., 2004, Volk and Todd, 2007). This is similar to the heritability estimated for ADHD symptoms based on parent and teacher reports (70-80%, see section 1.4) and indicates that individual differences in emotional lability are largely accounted for by genetic variation. Second, twin research more generally has linked ADHD to phenotypes resembling emotional lability, with moderate to strong genetic correlations between ADHD and ODD (r_G = 0.95, Wood et al., 2009a), ADHD and depression (r_G = 0.67 for girls, r_G = 0.77 for boys, Cole et al., 2009), and ADHD and borderline personality disorder (r_G = 0.70, Distel et al., 2011). One inference is that ADHD will also share genetic influences with the specific symptoms of emotional lability, although this has yet to be addressed.

1.8.6 Molecular genetic studies of emotional lability and ADHD

Few molecular genetic studies of emotional lability in ADHD have been conducted. One recent study (Robison et al., 2010) examined eight candidate genes in relation to emotional lability (DAT21, 5HT1B, BDNF, HRT2A, SNAP25, COMT and MAOA). One SNP for the gene 5HT1B was significantly associated with the presence of emotional lability but not with total ADHD symptom scores. This result did not survive correction for multiple testing, but is nonetheless similar to a previous study indicating an association of 5HT1B with impulsivity and aggression (Zouk et al., 2007). Another study found evidence of an association between DAT1 and emotional lability (Gruber et al., 2009). No genome-wide analyses of emotional lability have yet been conducted; however a family-based GWAS of DESR, assessed using the CBCL in children, failed to identify any genome-wide significant results (Mick et al., 2011). This study was likely underpowered with a sample size of only 341 probands from 339 trios. Further molecular genetic research into the association between ADHD and emotional lability symptoms is therefore required.

1.9 AIMS OF THESIS

The present thesis tackles several gaps in the existing literature on ADHD to address four main aims.

1.9.1 Aim 1: Understand rater effects in twin studies of ADHD

The first aim was to understand the impact of rater effects in twin studies of ADHD. As observed in section 1.4.5, heritability estimates vary as a function of how ADHD symptoms are assessed. The twin study in chapter 3 therefore compares genetic influences on parent, teacher and self-ratings of ADHD symptoms.

1.9.2 Aim 2: Explore the phenotypic and aetiological associations between ADHD and temperament

The second aim was to examine the association between ADHD and temperament in adults. The literature reviewed in section 1.7 identifies substantial phenotypic associations between ADHD and novelty seeking, yet the underlying aetiology of this association has remained unknown. This is addressed in chapter 4.

1.9.3 Aim 3: Examine the relationship between symptoms of ADHD and emotional lability

The third aim concerns the aetiological relationship between ADHD and emotional lability. As reviewed in section 1.8, there is now mounting evidence of a clinical association between ADHD and emotional lability symptoms. Chapter 5 therefore examines the extent to which there are genetic and environmental associations between ADHD and emotional lability symptoms, while chapter 6 explores the association of ADHD and emotional lability with cognitive performance in a community-based sample.

1.9.4 Aim 4: Test the polygenic theory of ADHD

The fourth aim was to test the polygenic theory of ADHD. As indicated in section 1.6, molecular genetic studies have generally failed to identify markers that explain a significant proportion of the variance in ADHD. The research in chapter 7 therefore applies the polygenic profile score method to predict ADHD affection status in a sample of ADHD probands, and to predict ADHD quantitative trait scores, symptoms of emotional lability and cognitive performance within a sample drawn from the general population.

2. METHODOLOGY

2.1 OVERVIEW

This chapter begins by summarising the samples and measures used in the research reported in chapters 3 to 7. It then explains the twin method, its assumptions and statistical procedures. It finally explains the polygenic method.

2.2 SAMPLES AND MEASURES

2.2.1 TEDS (used in chapters 3 and 7)

2.2.1.1 Sample

The Twins Early Development Study (TEDS) is a longitudinal, population-based twin cohort from the United Kingdom (UK) (Oliver and Plomin, 2007, Trouton et al., 2002). Ethical approval was obtained from the Research Ethics Committee of the Institute of Psychiatry, King's College London, UK. Families were recruited via the Office of Population Census and Surveys (now the Office for National Statistics, ONS), who contacted all traceable mothers of live-born twins in England and Wales in the years 1994 to 1996 (N = 16,810 families). A total of 16,302 families were subsequently invited to participate in the first wave of data collection, of whom 13,732 returned completed questionnaires (response rate = 84.2%). The cohort has since been followed prospectively, with some or all families invited to participate when twins were aged approximately 2, 3, 4, 7, 9, 10, 12, 14 and 16 years. The primary form of data collection was via postal questionnaire; augmented with home-visits, telephone interviews and/or internet-based testing across different waves of data collection.

In the years 2007-2009, DNA was collected via cheek swab for one twin per pair from 3,747 families for use in genome-wide analyses. Families were selected for inclusion based on a high response rate at previous data collection points, with DNA taken from the twin with the most complete set of phenotypic data available. Zygosity was determined via a postal questionnaire shown to

have 95% accuracy when compared to zygosity assignment based on DNA (Price et al., 2000).

The phenotypic data used in chapters 3 and 7 were collected in 2005-2007, when twins were approximately 12 years of age (range = 11-12 years). A total of 8,438 families were contacted, of whom 7,519 (89.1%) responded. This constitutes 46.9% of the sample invited to participate at the first wave of data collection. Non-contact was due to withdrawal from the study, inactivity at previous data collection points, non-participation in recent studies, and medical exclusions (see criteria below). Full attrition analyses have not been conducted because not all families were invited to take part on all testing occasions; however, available analyses indicate that TEDS families are representative of the UK population with respect to ethnicity, education level and employment status, including at age 12 (Dale et al., 2010, Oliver and Plomin, 2007). More recent analyses additionally indicates some selective attrition: respondents at age 12 scored significantly lower for ADHD symptoms than did non-respondents, based on the data from age 7 (Greven et al., 2011c).

Prior to the data analyses in chapters 3 and 7, standard exclusion criteria were applied. Families were excluded if one or both twins suffered from a specific medical syndrome, (chromosomal abnormalities, cerebral palsy, cystic fibrosis, profound deafness, complete blindness, organic brain damage, autism spectrum disorders, global developmental delay), if either twin had died, or if there had been pre/perinatal complications (low birth weight or gestational age, heavy drinking during pregnancy, a long period of hospitalisation after birth). Those with an unknown or uncertain zygosity were also excluded. For the polygenic analyses in chapter 7, those with genome-wide genotype data that failed standard quality control (QC) procedures were additionally excluded (see section 2.4.2.2). Following all exclusions, the twin model-fitting analyses in chapter 3 included 12,581 individuals from 6,372 families. The polygenic analyses in chapter 7 included 3,152 individuals (one twin per family).

2.2.1.2 Measures

The main measure used in chapter 3 was the Strengths and Difficulties Questionnaire (SDQ, Goodman, 2001), completed via post by parents, teachers and the twins themselves. The SDQ is a 25-item questionnaire designed to measure common mental health problems during childhood and adolescence. ADHD symptoms were assessed using the SDQ hyperactivity scale, a five-item measure of inattention *("easily distracted, concentration wanders", "sees tasks through to the end, good attention span"*), hyperactivity (*"restless, overactive, cannot stay still for long", "constantly fidgeting or squirming"*) and impulsivity ("*thinks things out before acting"*). There are insufficient items to provide a valid separation of the inattentive and hyperactive/impulsive symptoms into separate subscales and the loading of all five items onto a single scale is supported by factor analyses (Goodman, 2001, Van Roy et al., 2008). Each item was rated on a 3-point Likert scale scored 0-2, averaged to generate a total score. A minimum 3 out of 5 items had to be non-missing for inclusion in analyses.

Parent ratings were available for 11,178 twins from 5,590 pairs (2 incomplete pairs), teacher ratings for 9,365 twins from 5,217 pairs (1,069 incomplete), and self-ratings for 11,158 twins from 5,621 pairs (84 incomplete). Of the teacher ratings, 3,720 were completed by the same teacher for each twin from a pair (1,868 pairs, of which 16 incomplete), while 5,645 were completed by different teachers (3,349 pairs, of which 1,053 incomplete). Internal consistency (Chronbach's Alpha, α) was 0.76 for parent ratings, 0.86 for teacher ratings (same-teacher α = 0.87, different-teacher α = 0.84) and 0.69 for self-ratings. This is generally consistent with the results of prior research attributing sound psychometric properties to the SDQ (Goodman, 2001).

Data from the SDQ hyperactivity scale was also used in chapter 7, but only for those individuals with post-QC genome-wide genotype data available. This included 2,694 parent ratings, 2,138 teacher ratings and 2,691 self-ratings, with highly similar internal consistencies to those reported above (parent α = 0.77, teacher α = 0.86, self-rating α = 0.69).

The Conners' Parent Rating Scale – Revised (CPRS-R, Conners et al, 1998a) was also used in chapter 7, to assess ADHD symptoms in accordance with DSM-IV (see section 1.2), completed via post. The CPRS-R includes 9 inattentive and 9 hyperactive-impulsive items, rated on a 4-point Likert scale scored 0-3. Items were averaged to create hyperactive-impulsive and inattentive symptom scores, and a total ADHD symptom score. Scores for hyperactivity-impulsivity and inattention were only generated if at least 5 out of 9 items from the respective scales were non-missing. A score for total ADHD was only generated if at least 9 out of 18 items were non-missing. Thus, of the twins with post-QC genotype data, 2,692 had a CPRS-R score for hyperactivity-impulsivity, 2,695 had a score for inattention and 2,693 had a score for total ADHD. Prior analyses have indicated good psychometric properties for the CPRS-R (Conners et al., 1998a). Internal consistencies in this sample were α = 0.83 (hyperactivity-impulsivity), α = 0.90 (inattention) and α = 0.91 (total ADHD).

2.2.2 TCHAD (used in chapter 4)

2.2.2.1 Sample

The Swedish Twin Study of Child and Adolescent Development (TCHAD) is a longitudinal, population-based twin cohort from Sweden (Lichtenstein et al., 2007). Ethical approval was obtained from the Ethics Committee of the Karolinska Institutet, Stockholm, Sweden. Recruitment was via birth records, with the families of all twin pairs born in Sweden between May 1985 and December 1986 invited to take part (N = 1,489). Of these, 1,339 families participated in the first wave of data collection in 1994 when twins were aged 8-9 years, giving a response rate of 89.9%. The cohort has since been followed prospectively, with data collected when twins were aged 13-14, 16-17, 19-20, and 24-25 years. Data collection was via postal questionnaire.

Twin zygosity was determined via DNA testing: Twins' DNA was extracted from saliva samples, using OraGene® DNA (DNA Genotek Inc., Ontario, Canada) self-collection kits. For twins without a DNA sample, zygosity was determined based on an algorithm derived from discriminant analyses of twins' and parents' responses to validated (95% accurate) questionnaires (Lichtenstein et al., 2007,

Tuvblad et al., 2011). In cases of any contradictions between zygosity assignments, the zygosity was set to unknown, and the twins were excluded from the analyses (N = 100). Consistent with prior conventions, this was the only exclusion criterion applied, since medical exclusions took place during recruitment (Lichtenstein et al., 2007, Tuvblad et al., 2011, Chang et al., 2013).

The phenotypic data used in chapter 4 were from the fourth wave of data collection, conducted in 2005 when twins were aged 19-20 years. All families were invited to participate, with the mothers and fathers of twins approached separately, in addition to the twins themselves. Responses were received from at least 1 of the parents for 1,158 twins and via self-report from 1,705 twins. Analyses indicate that the families participating in TCHAD are representative of the Swedish population with regard to educational level and employment status but not ethnicity, with participating families more likely to come from ethnically homogeneous neighbourhoods (Lichtenstein et al., 2007). There is also some evidence of selective attrition: respondents at wave 4 scored significantly lower for ADHD symptoms and were more likely to be female (Chang et al., 2013, Larsson et al., 2011).

2.2.2.2 Measures

The measures used in chapter 4 were self-report questionnaires completed by the twins themselves. Respondents were only included in analyses if they had complete data available for one or more of these measures, giving a maximum sample size of 1,634 twins from 868 pairs (102 incomplete pairs).

ADHD symptoms were assessed using an 18-item questionnaire based on the full set of symptoms listed in DSM-IV (see section 1.2). Items were rated on a 3-point Likert scale scored 0-2, summed to create total scores for hyperactivity-impulsivity (9 items) and inattention (9 items). Internal consistencies were $\alpha = 0.79$ and $\alpha = 0.76$, respectively.

Temperament was assessed using a shortened version of the Temperament and Character Inventory (TCI, Cloninger et al., 1993). The TCI assessed temperament across four separate dimensions, generally supported via factor

analyses and psychometric assessment in earlier research (see section 1.7). The shortened version of this scale included 60 temperament items rated as "true" or "false". Responses were coded as 1 or 2 and summed to generate total scores for each dimension, such that higher scores indicated greater scores for each respective dimension. The first dimension was novelty seeking, which measured exploratory excitability, impulsiveness, extravagance and disorderliness across 20 items (e.g. *"When nothing new is happening, I usually start looking for something that is thrilling or exciting"*). The second dimension was harm avoidance, which measured anticipatory worry, fear or uncertainty, shyness and fatigability across 20 items (e.g. *"When I have to meet a group of strangers I am more shy than most people"*). The third dimension was reward dependence, which measured sentimentality, attachment and dependence across 15 items (e.g. *"I like to please other people as much as possible"*). The fourth dimension was persistence, which measured eagerness of effort, ambition and perfectionism across 5 items (e.g. *"I am usually so determined that I continue to work long after other people have given up"*). Internal consistencies were $\alpha = 0.70$ (novelty seeking), $\alpha = 0.68$ (harm avoidance), $\alpha = 0.60$ (reward dependence), and $\alpha = 0.62$ (persistence).

2.2.3 CASTANET (used in chapter 5)

2.2.3.1 Sample

The Cardiff Study of all Wales and North West of England Twins (CASTANET) is a population-based twin cohort from the UK (van den Bree et al., 2007). Ethical approval was provided by the North West Multi Centre Research Ethics Committee, UK. The first wave of data collection (years 1990-1993) was used to generate a list of all twin births in the Greater Cardiff area of Wales for the years 1976 to 1991. Recruitment was via Birth Registers and the UK National Health Service (NHS). The second wave of data collection (years 1996-1997) was used to expand the sample to include twin births in the whole of Wales plus the Greater Manchester area of England, also for the years 1976 to 1991. From these time points a register of around 6,000 families was generated. However, not all families were invited to participate in research: the first wave of data collection approached 376 families, of whom 287 (76.3%) took part; the second

wave approached 3,955 families, of whom 2,764 (69.9%) took part. The third (year 2000) and fourth (years 2004-2005) waves of data collection gathered a mix of new and follow-up data on twins aged 5-17 and 12-20 years, respectively. All data collection was via postal questionnaire. Zygosity was determined using an algorithm applied to the results of a twin similarity questionnaire (Cohen et al., 1975, Thapar and McGuffin, 1994), verified for a subset of twins using DNA (Payton et al., 2001).

The phenotypic data included in chapter 5 were collected at wave 2 from a subset of twins born in the Greater Manchester area between the years 1980-1991. A total of 3,089 families were initially identified, of whom 2,846 were invited to take part. Reasons for non-contact included untraceable addresses, health or social care problems that rendered contact inappropriate, emigration, and the death of one twin from a pair. Of the contacted families, 2,082 (73.2%) responded. Prior analysis indicates that the twins from non-respondent families were significantly younger than for respondents, and that respondents did not differ from the Greater Manchester population with regard to ethnicity or occupation (Thapar et al., 2000). Prior to data analyses those with an unknown or uncertain zygosity were also excluded, leaving a final sample size of 3,840 individuals from 1,920 twin pairs.

2.2.4.2 Measures

All measures used in chapter 4 were completed by the parents of participating twins, with complete data available for all respondents (i.e. for all 3,840 twins). ADHD symptoms were assessed using a modified version of the DuPaul Rating Scale (DuPaul, 1981). The original scale was devised to measure the 14 ADHD symptoms outlined in DSM-III-R, modified in this cohort to include 4 additional items in accordance with DSM-IV (Thapar et al., 2000). The scale thus included 18 ADHD items across two dimensions: hyperactivity-impulsivity (9 items) and inattention (9 items). Items were rated on a Likert scale scored 0-3, with responses summed to create a total score for each dimension. Higher scores indicated greater severity of symptoms. Internal consistency was $\alpha = 0.90$ for hyperactivity-impulsivity and $\alpha = 0.93$ for inattention.

Emotional Lability was assessed using the parent-rated Conner's 10-item scale (Conners et al., 1998a). Prior research has identified a two dimensional structure for the Conner's 10-item scale, with six items loading onto a Restless-Impulsive factor and four items loading onto an emotional lability factor (Parker et al., 1996, Westerlund et al., 2009). Accordingly, only four items (*"demands must be met immediately – easily frustrated", "cries often and easily", "mood changes quickly and drastically", "temper outbursts, explosive and unpredictable behaviour"*) were used as a measure of emotional lability. An exploratory factor analysis of the ADHD and emotional lability items in this sample identified a 3-factor solution, in which the 9 hyperactive-impulsive, 9 inattentive and 4 emotional lability items loaded onto three separate dimensions (Chen, unpublished data). The four emotional lability items were rated on a Likert scale scored 0-3. Responses were summed to generate a total score, where higher scores indicated greater symptom severity. Internal consistency was $\alpha = 0.82$.

2.2.4 SAIL (used in chapters 6 and 7)

2.2.4.1 Sample

The Study of Activity and Impulsivity Levels in children (SAIL) is a population-based twin cohort from the UK (Kuntsi et al., 2006). Ethical approval was obtained from the Research Ethics Committee of the Institute of Psychiatry, King's College London, UK. Participants were recruited via TEDS (see section 2.2.1). Families suitable for inclusion were identified based on the following criteria: twins' birthdates between 1st September 1995 and 31st December 1996; living within feasible traveling distance of the Research Centre (i.e. return day trip); ethnic origin of white European (to reduce population heterogeneity for molecular genetic studies); recent participation in TEDS (i.e. return of questionnaires at either the 4 or 7 year data collection points); no extreme pre/perinatal difficulties; no specific medical syndromes or chromosomal anomalies; not participating in other TEDS sub-studies; and not on stimulant or other neuropsychiatric medications. This led to the identification of 1,230 suitable families, of whom 672 (55%) agreed to participate. Zygosity assignments were taken from TEDS.

An additional 32 individuals were excluded based on the following criteria: IQ below 70; mild autism; epilepsy; illness on the day of testing; and one each due to obsessive-compulsive disorder, neurofibromatosis, cerebral palsy, hyperthyroidism, severe autism, and receipt of stimulant medication for ADHD. The final sample thus comprised 1,312 individuals from 668 twin pairs (24 incomplete pairs) included in the modelling presented in chapter 6. Of these, 330 individuals (one twin per family) had genome-wide genotype data that passed QC and were thus suitable for inclusion in the polygenic analyses in chapter 7.

2.2.4.2 Measures

Table 2.1 Number of twins with data available in SAIL

	Used in chapter 6		Used in chapter 7
	N twins	N pairs (N incomplete)	N twins (1 per family)
IQ	1309	668 (27)	324
DSF	1309	668 (27)	-
DSB	1309	668 (27)	-
MRT	1247	666 (85)	-
RTV	1247	666 (85)	315
CE	1290	667 (44)	320
CI	1223	628 (33)	-
HI	1159	611 (63)	-
IA	1159	611 (63)	-
EL	1155	906 (63)	287

Note: DSF = digit span forward; DSB = digit span backward; MRT = mean reaction time; RTV = reaction time variability; CE = commission errors; CI = choice impulsivity; HI = composite rating of hyperactivity-impulsivity; composite rating of IA = inattention; composite rating of EL = emotional lability.

The phenotypic data used in chapters 6 and 7 were derived from cognitive and behavioural measures (Kuntsi et al., 2006). The number of twins with complete data available are summarised in Table 2.1. Cognitive assessment took place at the research centre (MRC Social, Genetic and Developmental Psychiatry Centre, King's College London). Two testers assessed the twins from each pair simultaneously in separate testing rooms. Tasks were administered in a fixed

order as part of an extensive testing session lasting approximately 2.5 hours (including breaks).

The Wechsler Intelligence Scales for Children, Third Edition (WISC-III) (Wechsler, 1991): The vocabulary, similarities, picture completion and block design subtests from WISC-III were used to obtain an estimate of the child's IQ [pro-rated following established procedures (Sattler, 1992)]. Digit span forwards and backwards were included as measures of short-term and working memory.

The Go/No-go task (Borger and van der Meere, 2000, Kuntsi et al., 2005a, van der Meere et al., 1995): On each trial, one of two possible stimuli (X or O) appeared for 300 milliseconds (ms) in the middle of a computer screen. The child was instructed to respond only to the *'go'* stimuli (X) and to react as quickly as possible, but to maintain a high level of accuracy. The proportion of *'go'* stimuli to *'no-go'* stimuli was 4:1. There were three conditions and a practice session preceded each experimental condition. The slow condition had an inter-stimulus interval (ISI) of 8 seconds (s) and consisted of 72 trials. The fast condition consisted of 462 trials and had an ISI of 1s. The order of presentation of the slow and fast conditions was varied randomly across children. An incentive condition, which rewarded fast, correct responses, was always administered last to prevent adverse effects on performance in non-rewarded conditions. Performance under the incentive condition was not of interest in this thesis; thus a detailed description can be found elsewhere (Kuntsi et al., 2009). The response variables obtained were the mean reaction time (MRT) to *go* stimuli, reaction time variability (RTV, i.e. the standard deviation of RTs), commission errors (CE, i.e. number of incorrect responses to the *no-go* stimulus) and omission errors (OE, i.e. failures to respond to the *go* stimulus).

The Fast task (Andreou et al., 2007, Kuntsi et al., 2005a, Kuntsi et al., 2006): The baseline condition (72 trials) followed a standard warned four-choice reaction time (RT) (Leth-Steensen et al., 2000). A warning signal (four empty circles, arranged side by side) first appeared on the screen. At the end of the fore period of 8s (presentation interval for the warning signal), the circle designed as the target signal for that trial was filled (coloured) in. The child was asked to make a compatible choice by pressing the response key that directly

corresponded in position to the location of the target stimulus. Following a response, the stimuli disappeared from the screen and a fixed inter-trial interval of 2.5s followed. Speed and accuracy were emphasised equally. If the child did not respond within 10s, the trial terminated. The baseline condition was preceded by a practice session, during which the child had to respond correctly to five consecutive trials. It was followed by a fast/incentive condition. Because performance under this latter condition was not of interest, a detailed description can be found elsewhere (Kuntsi et al., 2009). The response variables were MRT and RTV for the number of correct responses at baseline.

The Maudsley Index of Delay Aversion (Kuntsi et al., 2006, Kuntsi et al., 2001b, Paloyelis et al., 2009): Two conditions, each with 20 trials, were administered. In each trial, the child had a choice between a smaller-immediate reward (one point involving a 2-second pre-reward delay) and a larger-delayed reward (two points involving a 30-second pre-reward delay). In the no post-reward delay condition, choosing the small reward led immediately to the next trial, reducing the overall length of the condition. In the post-reward delay condition, choosing the small reward led to a delay period of 30 seconds, and choosing the large reward led to a delay period of 2 seconds before the next trial; therefore, the overall delay was constant and independent of choice made. The order of conditions was randomly chosen for each twin. The response variable was choice impulsivity (CI), defined as the percentage of small-immediate reward choices in the no post-reward delay condition.

Derivation of composite variables: Data from the go/no-go task slow condition and the fast task baseline condition were summed to create composite measures of MRT and RTV. Prior analyses indicate that performance in both conditions is significantly associated with ADHD (Kuntsi et al., 2009) and support the use of composite scores to reduce measurement error (Kuntsi et al., 2006). A composite of CE across the Go/No-go task slow and fast conditions was similarly created by summing performance across conditions. OE were rare in this sample and therefore not included in analyses, in line with prior conventions (Kuntsi et al., 2006, Kuntsi et al., 2009).

Behavioural ratings were obtained from the parents of twins at the time of cognitive assessment, using the Long Version of Conners' Parent Rating Scale (CPRS-R:L; Conners et al. 1998a). Parents were also asked for consent to obtain behavioural ratings from the teachers of twins, who completed the Long Version of Conners' Teacher Rating Scale (CTRS-R:L; Conners et al. 1998b) via post. For some twins the parent and teacher data were only partially complete. Where this occurred, missing data were pro-rated (i.e. a summary score generated based on the mean of individual questions on the rest of the subscale) if there was more than 75% completion for each subscale (parent pro-ratings for 13-18 individuals; teacher pro-ratings for 18-26 individuals). This is consistent with scoring recommendations (Conners, 1997). Parent data were completely missing for two twins from one family, while teacher data were completely missing for 151 twins from 104 families. Due to the small number of items for the emotional lability scale, parent ratings for 3 individuals and teacher ratings for 1 individual could not be pro-rated and were coded as missing.

To assess ADHD symptoms, parent and teacher responses to the DSM-IV ADHD items were summed for hyperactivity-impulsivity (9 items) and inattention (9-items), creating two composite scales that reflected a pervasive view of symptoms across the respective dimensions. Parent and teacher scores from were also summed for the emotional lability scale, which included three items rated by parents and teachers (*"temper outbursts: explosive, unpredictable behaviour"*, *"cries often and easily"*, *"mood changes quickly and drastically"*) and one additional item rated by teachers only (*"demands must be met immediately – easily frustrated"*). The separation of emotional lability from hyperactivity-impulsivity and inattention has been documented previously (see section 2.2.3). Internal consistencies are not reported due to the use of multi-rater composites.

2.2.5 PGC (used in chapter 7)

The Psychiatric Genomics Consortium (PGC) was established in 2007 to facilitate international pooling of genome-wide genotype data for five psychiatrics disorders, including ADHD (Sullivan, 2010). The ADHD subgroup includes data from nine international samples examining the association of common single nucleotide polymorphisms (SNPs) with ADHD. Data from all

samples were included in the analyses in chapter 7, split into discovery and target sets. The discovery set comprised data from eight samples, used to generate a polygenic score for ADHD; the target set comprised data from one sample, used to test the polygenic score for association with ADHD affection status. Details of the methodology are set out in section 2.4.

2.2.5.1 PGC discovery set

Table 2.2 Summary of the eight PGC samples included in the polygenic discovery set

Sample (key reference)	N	Ethnicity	ADHD measure(s)
CHOP (US) (Elia et al., 2010)	Trios: 358	EU ancestry	KSADS-P-IVR
PUWMA (US) (Mick et al., 2010)	Trios: 702	EU ancestry	MAGIC, K-SADS-PL, K-SADS-E, SADS-LA
IMAGE 2 (DE, NL, ROI, UK, US) (Neale et al., 2010a)	Cases: 892 Controls: 7086	EU ancestry	PACS, K-SADS-PL, Kinder DIPS, CAPA
Canada (Lionel et al., 2011)	Trios: 170	EU ancestry	PICS
China (Yang et al., 2013)	Cases: 1014 Controls: 932	Han Chinese ancestry	CIDS-Chinese
Germany (Hinney et al., 2011)	Case: 495 Control: 1298	EU ancestry	K-SADS-PL
Spain (Ribasés et al., 2009)	Case: 616 Control: 435	EU ancestry	SCID-I & II, CAADID, K-SADS-PL
ROI/UK (Stergiakouli et al., 2012)	Case: 727 Control: 1801	EU ancestry	CAPA

Note: CHOP = Children's Hospital of Philadelphia; PUWMA = Pfizer-funded study from the University of California, Los Angeles, Washington University, and Massachusetts General Hospital; IMAGE 2 = International Multi-centre ADHD Genetics project 2; DE = Germany; NL = Netherlands; ROI = Republic of Ireland; UK = United Kingdom; US = United States; N gives number of probands from trios or number of ADHD cases and number of controls; EU denotes European ancestry; ADHD measures are the clinical interviews used to diagnose ADHD; KSADS-P-IVR = Schedule for Affective Disorders and Schizophrenia for School Age Children IV Revised (Ambrosini, 2000); MAGIC = Missouri Assessment of Genetics Interview for Children (Todd et al., 2003); K-SADS-PL = Schedule for Affective Disorders and Schizophrenia for School-Age Children – Present and Lifetime Version (Kaufman et al., 1997); K-SADS-E = Schedule for Affective Disorders and Schizophrenia for School-Age Children – epidemiologic version (Orvaschel, 1994); SADS-LA = Schedule for Affected Disorders and Schizophrenia — Lifetime Version updated for DSM-IV (Fyer et al., 1995); PACS = Parental Account of Childhood Symptoms (Chen and Taylor, 2006); Kinder DIPS = Diagnostic Interview for Children and Youth (Schneider et al., 2009); CAPA = Child and Adolescent Psychiatric Assessment (Angold and Costello, 2000); PICS = Parent Interview for Child Symptoms (Ickowicz et al., 2006); CIDS-Chinese = Chinese version of the Clinical Diagnostic Interview Scale (Yang et al., 2004); SCID-I & II = Structured Clinical Interview for DSM-IV Axis I and II Disorders (First et al., 1997, First et al., 2002); Conners' Adult ADHD Diagnostic Interview for DSM-IV (Epstein et al., 1999).

The eight samples included in the discovery set are summarised in Table 2.2. Three samples included family-based genomic data from ADHD parent-proband trios, while the remaining five samples included population-based genomic data for ADHD cases and controls. The purpose of the discovery set was to generate a polygenic score for ADHD using the largest possible dataset; thus only basic information on the number of cases and controls with data that passed QC is presented in Table 2.2. Details of the QC procedures and polygenic analyses are provided in section 2.4.

All probands included in analyses met DSM-IV diagnostic criteria for ADHD and were screened free from low IQ, neurological disorders and other factors that may have biased results. Control participants were healthy but unselected and thus not screened free from ADHD. Some controls were recruited as part of individual studies while other controls were recruited from the wider PCG (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). Full details on ascertainment and sample characteristics are available via the key references in Table 2.2. This includes information on the ethics procedures of individual studies, which conformed to the Declaration of Helsinki (World Medical Association, 2008). DNA samples were from blood or saliva.

2.2.5.2 PGC target set

One sample comprised the independent target set: the International Multi-centre ADHD Genetics project (IMAGE) (Neale et al., 2008). IMAGE was selected as the target set since it includes detailed data on a number of behavioural and cognitive phenotypes (see section 1.6.3). Familial data were collected from 11 clinical centres across eight European countries (Belgium, Germany, Holland, Israel, Republic of Ireland, Spain, Switzerland and UK). Ethical approval was obtained from the respective Ethics Review Boards within each country. The full IMAGE sample includes phenotypic data for 1,404 ADHD probands from as many families, in addition to 1,828 siblings of probands. Data were collected when probands and siblings were aged 5-17 years, obtained while probands were off medication prescribed for the treatment of ADHD

wherever possible. Genome-wide genotype data were available for 958 affected proband-parent trios using DNA collected from blood.

Standard IMAGE exclusion criteria were applied to remove from analyses individuals with autism, epilepsy, an IQ below 70, brain disorders, and any genetic or medical disorder associated with externalising behaviours that might mimic ADHD. For the purposes of this thesis, three additional criteria were applied. First, only the designated ADHD proband was included in analyses, even in families where siblings also met diagnostic criteria and had genotype data available. This was to prevent inflation of the polygenic score. Second, only ADHD probands with a confirmed diagnosis of combined-type ADHD were included in analyses, based on prior research indicating that it might represent a genetically homogeneous ADHD subtype (Todd et al., 2001). Third, only those probands whose genotype data passed stringent QC procedures were included in analyses (see section 2.4). Following all exclusions, the final sample included 783 ADHD probands.

ADHD diagnoses were made using the Parental Account of Childhood Symptoms (PACS) (Chen and Taylor, 2006), a standardised diagnostic interview schedule used to assess for ADHD and other psychiatric disorders of childhood in accordance with DSM-IV. Diagnoses were verified using ADHD symptom data from behavioural rating scales including the CPRS-R:L and the CTRS-R:L (see descriptions of these measures in section 2.2.4).

2.3 TWIN ANALYSES

2.3.1 The twin method

The twin method is used to decompose phenotypic variance/covariance into genetic and environmental components for monozygotic (MZ) and dizygotic (DZ) twin pairs (Plomin et al., 2008). Twins can be similar due to shared genetic or shared environmental effects; in contrast, unique effects contribute to twin dissimilarity. In the classical twin method these effects are represented by four latent variance components (Rijsdijk and Sham, 2002): The additive genetic component (A) represents the cumulative effect of individual alleles; the non-

additive genetic component (D) represents interactions between alleles at the same or different loci (genetic dominance or epistasis); the shared environment (C) represents environmental influences that act to increase similarity between twins from a pair; the non-shared environment (E) represents environmental influences that act to decrease phenotypic similarity. E additionally subsumes measurement error. Broad-sense heritability is the sum of A+D.

2.3.2 Twin correlations and Falconer's equation

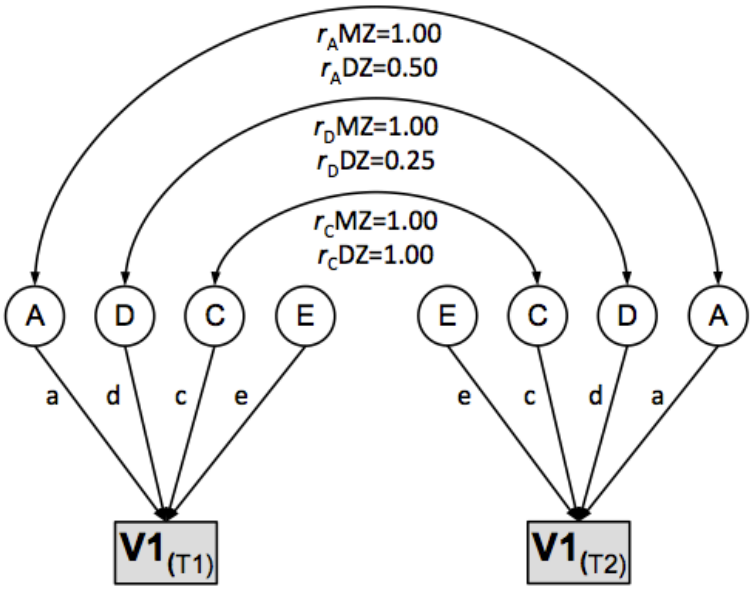
A simple method of estimating genetic/environmental influences is to examine twin correlations. It is assumed that additive genetic and non-additive genetic correlations within MZ twin pairs (r_AMZ, r_DMZ) are 1.00 respectively, since 100% of their genetic variation is shared. In contrast, within DZ pairs, the additive genetic correlation (r_ADZ) is assumed to be 0.50 and non-additive genetic correlation (r_DDZ) 0.25, reflecting on average 50% additive genetic similarly and 25% non-additive genetic similarity. Within MZ and DZ pairs 100% of shared environmental influences are in common, giving a shared environmental correlation (r_CMZ, r_CDZ) of 1.00, respectively. Non-shared environmental influences are unique to individuals and thus uncorrelated.

Accordingly, for a phenotype that is strongly influenced by A, the MZ cross-twin within-trait (r_{MZ}) correlation should be twice the size of the DZ (r_{DZ}) correlation. An imperfect MZ correlation indicates that there are E influences. MZ correlations more than twice the size of DZ correlations implicate D, while MZ correlations less than twice the size of DZ correlations implicate C. These principles can similarly be applied to interpret cross-twin cross-trait correlations, providing information about the genetic/environmental influences on covariation between different phenotypes (see section 2.3.7). A limitation of the classical twin design is that D and C are confounded, meaning that they cannot be modelled simultaneously. The pattern of twin correlations is therefore used to determine whether to model D or C.

To obtain an estimate of broad-sense heritability (h^2) from cross-twin within-trait correlations, Falconer's equation can be applied. The formula is: $h^2 = 2(r_{MZ} - r_{DZ})$. Influences of C are calculated as: $c^2 = r_{MZ} - h^2$. Influences of E are

calculated as: $e^2 = 1 - h^2 + c^2$. However, a limitation of this approach is that it cannot be used to adequately test for aetiological sex differences (see section 2.3.6) or the multivariate association between phenotypes (section 2.37); consequently, most twin analyses are implemented via structural equation models using maximum likelihood estimation (Rijsdijk and Sham, 2002).

Figure 2.1 Path diagram depicting genetic/environmental parameters



Legend: V1 = variable 1 for twin 1 (T1) or twin 2 (T2); a = parameter estimate for loading of A onto V1; d = parameter estimate for loading of D; c = parameter estimate for loading of C; e = parameter estimate for loading of E; r_A = additive genetic correlation between T1 and T2, set to 1.00 for MZ twins and 0.50 for DZ twins; r_D = non-additive genetic correlation, set to 1.00 for MZ twins and 0.25 for DZ twins; r_C = shared environmental correlation, set to 1.00 for MZ and DZ twins; E is uncorrelated across twins; note that D and C cannot be modelled simultaneously in the classical twin design; figure adapted from Rijsdijk and Sham (2002).

2.2.3 Path diagrams

Path diagrams provide a means of visualising variance/covariance, first introduced by Sewell Wright (Wright, 1921). An example for a single phenotype is presented in Figure 2.1. In this diagram, observed variables are depicted as rectangles, unobserved (latent) variables as circles, causal paths as single-headed arrows, and correlations as double-headed arrows. This is a standard method of presentation (Rijsdijk and Sham, 2002). Path tracing can be applied to calculate variance/covariance within a twin pair. For example, the covariance due to A can be calculated as *a*1*a* for MZ twins, or *a*0.5*a* for DZ twins. The

covariance due to D or C can be similarly derived. Three rules underpin path tracing: First, having progressed forward along a path, you cannot go back along the same path; second, each variable can only be passed once; third, only one path per trace can be represented by a double-headed arrow.

2.3.4 Structural equation models

A mathematically equivalent method of representing the variance/covariance structure depicted in path diagrams is to use structural equation models (SEMs). SEMs test specific hypotheses about the relationship between observed and latent variables (Rijsdijk and Sham, 2002). Variance/covariance matrices are fit to observed data via iterative processes, used to generate parameter estimates for latent variables that correspond to path coefficients (i.e. *a, d, c, e* in Figure 2.1). A key strength of this approach is that the fit of different SEMs can be compared to understand the aetiological contributions to phenotypic variance/covariance.

Throughout this thesis all SEMs were fit in Mx (Neale et al., 2006). Mx uses full-information maximum-likelihood (FIML) to obtain optimised parameter estimates that best fit the observed data (Neale et al., 2006, Rijsdijk and Sham, 2002). An advantage of the FIML approach is that it allows for estimation of parameters from missing data structures under normal theory (i.e. assuming a missing at random structure), meaning that data from incomplete twin pairs can be analysed. The significance and accuracy of parameter estimates is determined using likelihood-based 95% confidence intervals, whereby a parameter is progressively moved away from its FIML estimate in either direction until a significant deterioration in fit occurs (Neale and Miller, 1997). Confidence intervals that bound zero indicate that a parameter estimate is non-significant.

The significance of parameter estimates can additionally be determined by comparing full and restricted models. Restricted models are those that constrain parameter estimates from the full model (e.g. constraining a parameter to zero). Restricted models provide a more parsimonious solution to the data, but typically lead to deterioration in overall model fit. A significant deterioration in fit indicates that a restricted model provides a worse account of the observed data

structure and should be rejected. Because restricted models are nested within full models the difference in fit can be assessed using likelihood ratio chi-square tests (χ^2): The difference in minus twice the log likelihood of the data (-2LL) for the full and restricted model is calculated and compared against a chi-square distribution, with degrees of freedom (df) equal to the difference in the number of parameters. Throughout this thesis the most parsimonious solution was sought when fitting models.

The fit of different classes of model can also be compared; however since different models are non-nested the χ^2 test is not appropriate. Mx generates standard fit indices that can be used to compare different models, two of which are used throughout this thesis: Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC). AIC takes into account the number of parameters estimated and favours more parsimonious models over more complex models. A difference in AIC (ΔAIC) ≤ 2 indicates weak support for the model with the lower value, while ΔAIC = 3-10 indicates a stronger preference and ΔAIC ≥ 10 indicates a substantially stronger preference (Wagenmakers and Farrell, 2004). BIC also favours parsimony, particularly when there are large sample sizes. A difference in BIC (ΔBIC) ≤ 2 indicates weak support for the model with the lowest value, ΔBIC = 2-6 indicates some support for the lower value, ΔBIC = 6-10 indicates strong support, and ΔBIC ≥ 10 indicates a very strong preference (Raftery, 1995).

Throughout this thesis all SEMs were fit to raw data, pre-processed to meet the following requirements. First, non-normal data were transformed to ensure that all variables were normally distributed, an assumption of Mx. Second, all variables were regressed on age and sex, with residuals taken forward for inclusion analyses. This standard procedure is applied because each twin from a pair is of the same age and, most often, the same sex. This can cause genuine effects of age and sex to go undetected, leading to inflated estimates of twin similarity and over-estimation of C (McGue and Bouchard Jr, 1984). Third, all variables for inclusion in analyses were saved as a *.dat* file with one data column per-twin, per-variable. Twin order was randomised to avoid birth order effects; the exception being for DZ opposite sex twin pairs, for whom the

male twin was always included first to enable sex-limitation modelling (see section 2.3.6).

All data preparation was conducted using Stata version 10.1 (StataCorp., 2007). Stata was additionally used to conduct preliminary analyses, such as testing for mean differences by sex. Such analyses were performed using robust cluster function in Stata. This uses Huber-White Sandwich estimators to generate standard errors that are robust to non-independence among observations derived from clustered data (e.g. from twin pairs, who cluster in families) (Williams, 2000). Robust standard errors can additionally withstand minor deviations from normality, outliers, and heteroscedasticity.

2.3.5 Saturated models

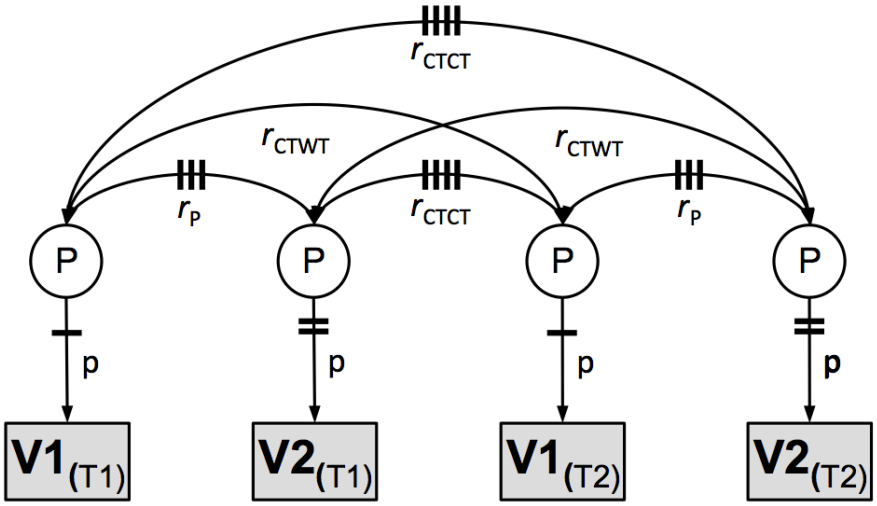
Saturated models are those that do not partition phenotypic variance/covariance into genetic and environmental components, and instead estimate the maximum number of means, variances and covariances across different sex-by-zygosity groups. Throughout this thesis, saturated models were fit for five sex-by-zygosity groups to allow tests of sex differences: MZ males, MZ females, DZ males, DZ females, and DZ opposite-sex twins. All cross-twin within-trait correlations, cross-twin cross-trait correlations and phenotypic correlations reported in chapters 3-6 were estimated using multivariate saturated models, constrained in the following ways (see bivariate example in Figure 2.2):

1. Models were constrained such that one set of means and variances was obtained for MZ males, MZ females, DZ males and DZ females (i.e. four sets of means and four sets of variances per phenotype)

2. Cross-twin cross-trait correlations were constrained to be equal within twin pairs, such that one set was obtained per sex-by-zygosity group

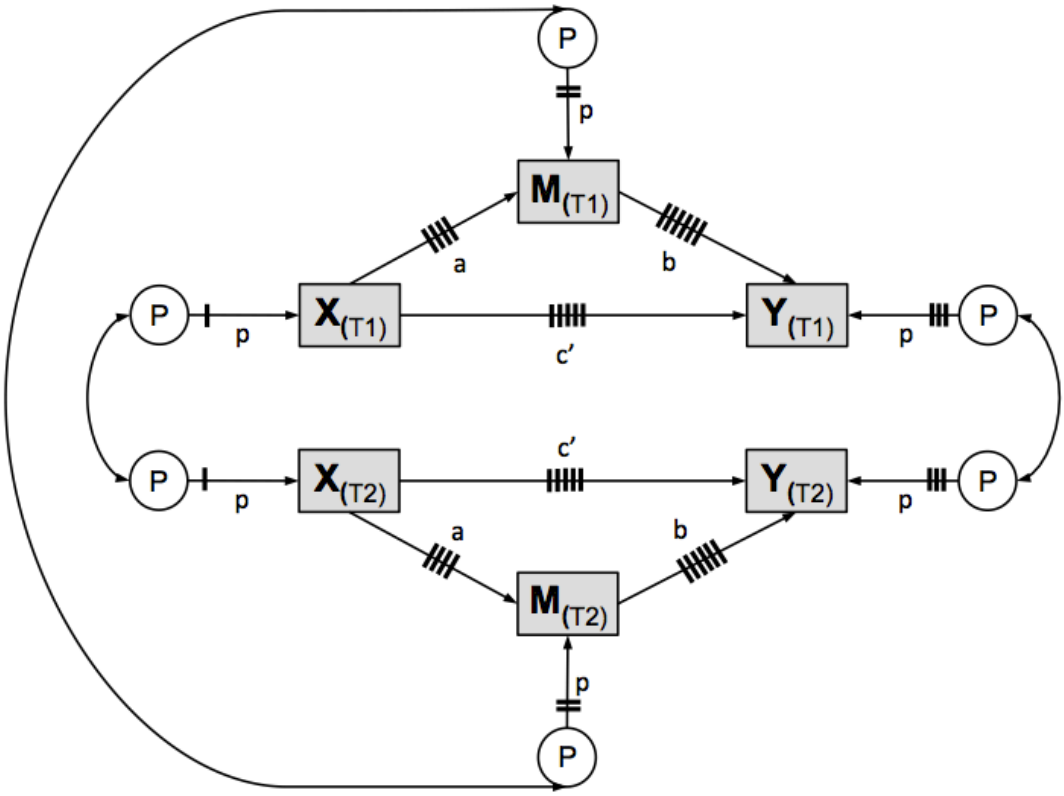
3. Phenotypic correlations were constrained to be equal across all sex-by-zygosity groups, such that one set of correlations was obtained for the entire sample

Figure 2.2 Path diagram for phenotypic correlations from the multivariate saturated model



Legend: V1 = variable 1, V2 = variable; T1 = twin 1, T2 = twin 2; P = phenotypic variance, calculated by tracing path p; r_P = phenotypic correlation; r_{CTWT} = cross-twin within-trait correlation; r_{CTCT} = cross-twin cross-trait correlation; parallel lines indicate parameter estimates constrained to be equal; this diagram provides a bivariate example for one sex-by-zygosity group.

Figure 2.3 Path diagram for the phenotypic mediation model



Legend: X = independent variable, M = mediator variable, Y = dependent variable, for twin 1 or 2 (T1, T2); P = phenotypic variance for X, M and Y, which correlate between T1 and T2; a = causal path between X and M; b = causal path between M and Y; c' = causal path between X and Y; parallel lines indicate parameter estimates constrained to be equal.

Additionally, in chapter 6, a phenotypic mediation model was fit to test for mediated associations between phenotypes. The model included causal phenotypic paths (a, b, c') between each pair of variables, based on Baron and Kenny's (1986) criteria for mediation (see section 6.3.2). These paths take the form of partial regression coefficients. The independent variable (X) thus accounts for a proportion of the variance in the mediator variable (M) via path a, and accounts for a proportion of the variance in the dependent variable (Y) via path c'. M additionally accounts for a proportion of the variance in Y via path b. A path diagram is depicted in Figure 2.3. An advantage of the mediation SEM over classical regression-based tests of mediation is the ability to estimate all paths simultaneously (Iacobucci, 2008).

2.3.6 Univariate models

2.3.6.1 Sex limitation models

Univariate models are used to decompose the variance for a single phenotype into the components A, D or C, and E. The univariate models fit throughout this thesis are full sex limitation models, used to test whether the genetic and environmental factors influencing males are different to those influencing females (qualitative sex differences), whether the magnitude of genetic/environmental factor loadings differs across sex (quantitative sex differences), and whether there are differences in phenotypic variances between males and females. The full sex limitation model (1) contains three nested (restricted) sub-models (2-4) and can be explained as follows:

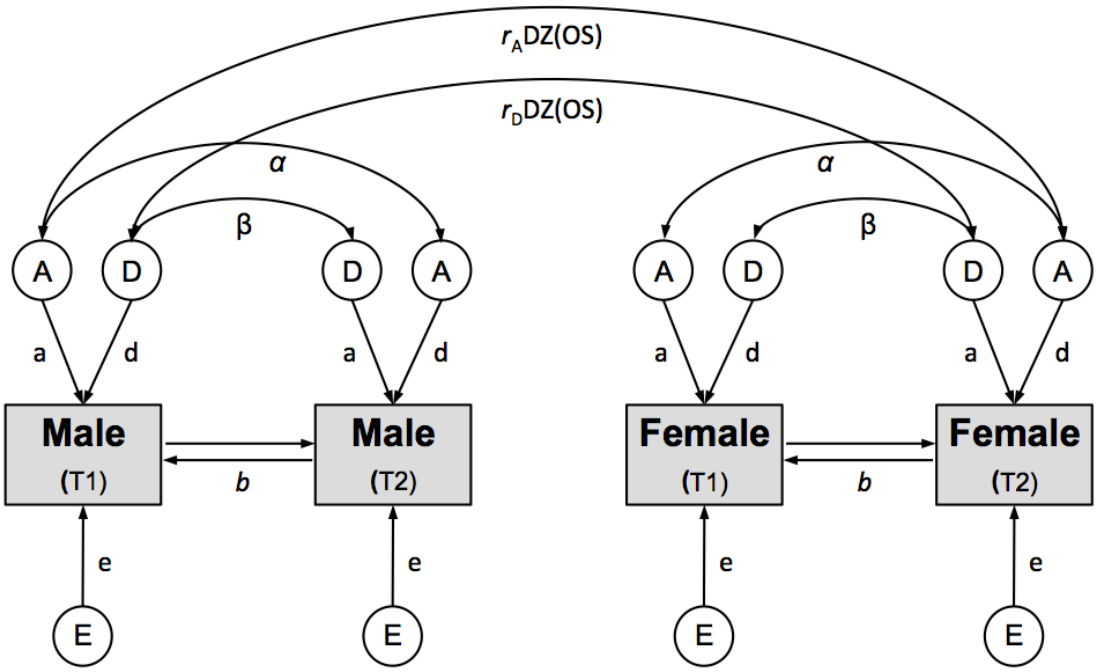
1. *The full sex limitation model* allows quantitative and qualitative differences in the parameter estimates between males and females, and freely estimates either r_A or r_D or r_C for DZ opposite-sex twins
2. *The common effects sex-limitation model* allows quantitative sex differences between males and females but no qualitative differences, fixing r_A to 0.5, r_D to 0.25 and/or r_C to 1.00 for DZ opposite-sex twins
3. *The scalar sex-limitation model* allows variance differences between males and females but no qualitative or quantitative differences, fixing r_A to 0.5, r_D to 0.25 and/or r_C to 1.00 for DZ opposite-sex twins and constraining the male

variance components to be a scalar multiple of the female variance components.

4. *The null model* equates all parameter estimates for males and females, testing the hypothesis that there are no sex differences.

A path diagram for the full sex-limitation model is presented in Figure 2.4. For illustrative purposes the diagram depicts A, D and E (not C). The choice on whether to parameterise D or C is made based on the pattern of twin correlations (see section 2.3.2). Should the pattern of correlations differ substantially between males and females, a hybrid model can be fit to the data, allowing D influences for males and C influences for females (or vice versa). This is plausible since D and C are not estimated simultaneously for the same twins. Once the best-fitting sex-limitation model is identified, variance components can be dropped until the most parsimonious solution is achieved.

Figure 2.4 Path diagram for the univariate full sex limitation model



Legend: V1 = T1 = twin 1, T2 = twin 2, with one male pair and one female pair; α = additive genetic correlation within male or female pairs, constrained to 1.00 for MZ twins and 0.5 for DZ twins; β = non-additive genetic correlation within male or female pairs, constrained to 1.00 for MZ twins and 0.25 for DZ twins; r_ADZ(OS) = additive genetic correlation for DZ opposite-sex pairs, where T1 is male and T2 is female, allowed to vary freely in the full sex limitation model but constrained to 0.50 for all sub-models; r_DDZ(OS) = non-additive genetic correlation for DZ opposite-sex pairs, allowed to vary freely in the full sex limitation model but constrained to 0.25 for all sub-models; b = contrast effect parameter.

2.3.6.2 Contrast effects

Low DZ correlations in the presence of significantly greater variances for DZ than MZ twin pairs are consistent with a contrast effect (Neale and Maes, 2004). Contrast effects are typically considered a form of rater bias (see section 1.4.6) that acts to reduce twin similarity, with a greater impact on DZ than MZ twin pairs. However, by including a contrast effect parameter (*b*) in SEMs the effect can be controlled for and its significance assessed. In the context of the full sex limitation modelling conducted in this thesis, *b* was initially parameterised separately for male, female and opposite-sex twin pairs. Tests of sex differences were then performed by equating the *b* parameter across sex-by-zygosity groups and assessing the change in model fit, conducted as an adjunct to the four-step sex-limitation model described above. For illustrative purposes, *b* is included in path diagrams; however contrast effects are only modelled when indicated by the pattern of twin variances and correlations.

2.3.7 Multivariate models

Multivariate models are used to decompose the covariance between different phenotypes into A, D or C, and E, based on cross-twin cross-trait correlations. Multivariate models can therefore be used to address two key questions. First, whether the same genetic/environmental influences operate across two or more phenotypes. Second, the extent to which the phenotypic correlation between variables is due to genetic versus environmental components. Univariate results were used to guide the multivariate modelling conducted throughout this thesis, including decisions on whether to parameterise C or D and/or *b*, and whether to incorporate sex differences into the models. For illustrative purposes, all path diagrams presented below depict A, D, E and *b.* Three classes of multivariate model were fit in chapters 3-5.

2.3.7.1 Cholesky decomposition

The Cholesky (triangular) decomposition parameterises the extent to which the genetic/environmental factors (A, D, E) loading onto one phenotype also load onto another. Because the Cholesky decomposition gives precedence to the

first variable (i.e. genetic/environmental factor loadings for the first variable account for some of the variance in all subsequent variables), it is recommended that the mathematically-equivalent correlated factors solution be interpreted when the order of variables is arbitrary (Loehlin, 1996). The correlated factors solution (Figure 2.5) parameterises the extent to which latent genetic/environmental factors (A, D, E) are correlated (r_A, r_D, r_E) across phenotypes. The Cholesky decomposition is the least restrictive multivariate model, since it makes no assumptions about the psychological mechanisms involved in phenotypic covariation. It can therefore be used as a baseline model against which to compare other models.

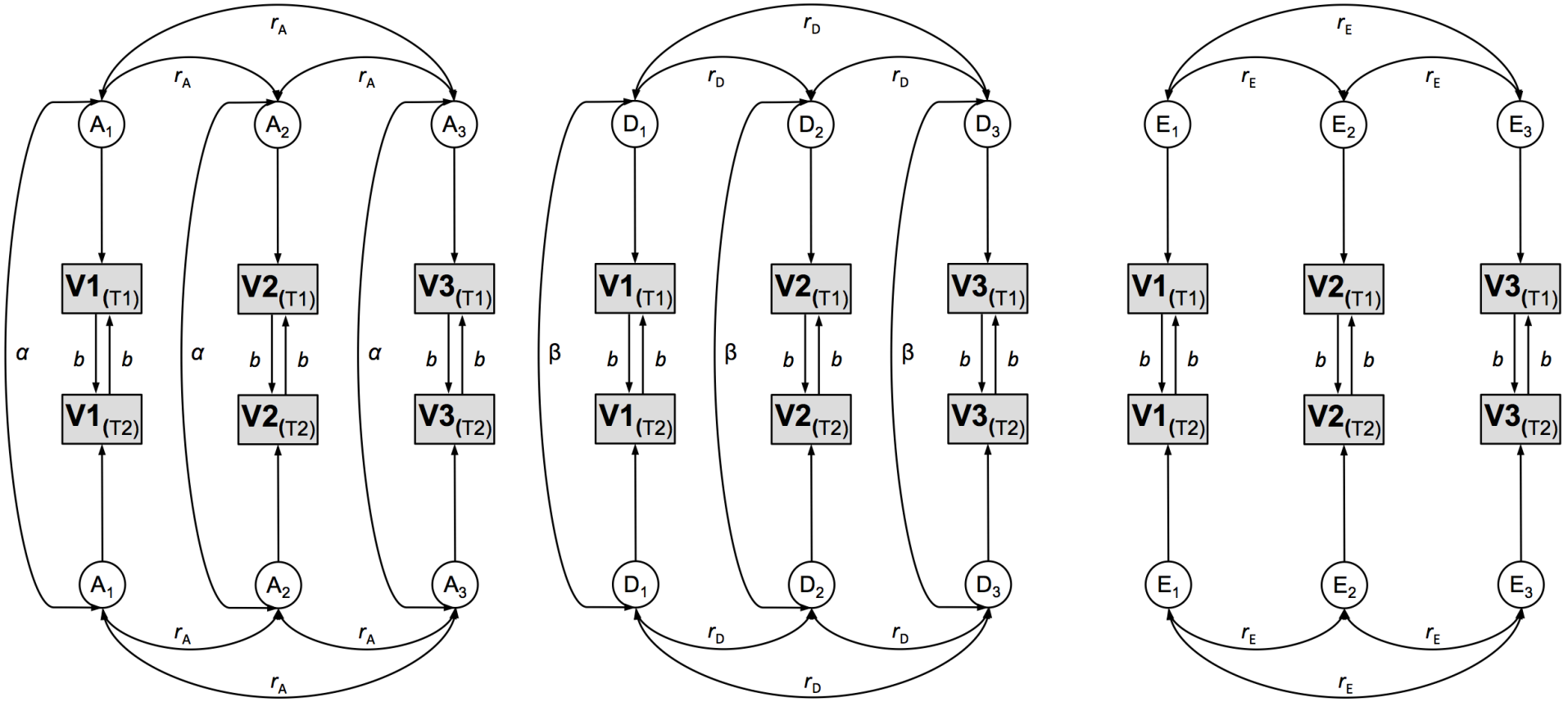
2.3.7.2 Independent pathway model

The independent pathway model (Figure 2.6) is based on a biometric model and assumes that phenotypic covariance is due to a single set of common genetic/environmental factors (A_C, D_C, E_C). These factors account for a proportion of the total variance in each phenotype. The remaining variance, which is unique to each phenotype, is accounted for by specific genetic/environmental factors (A_S, D_S, E_S).

2.3.7.3 Common pathway model

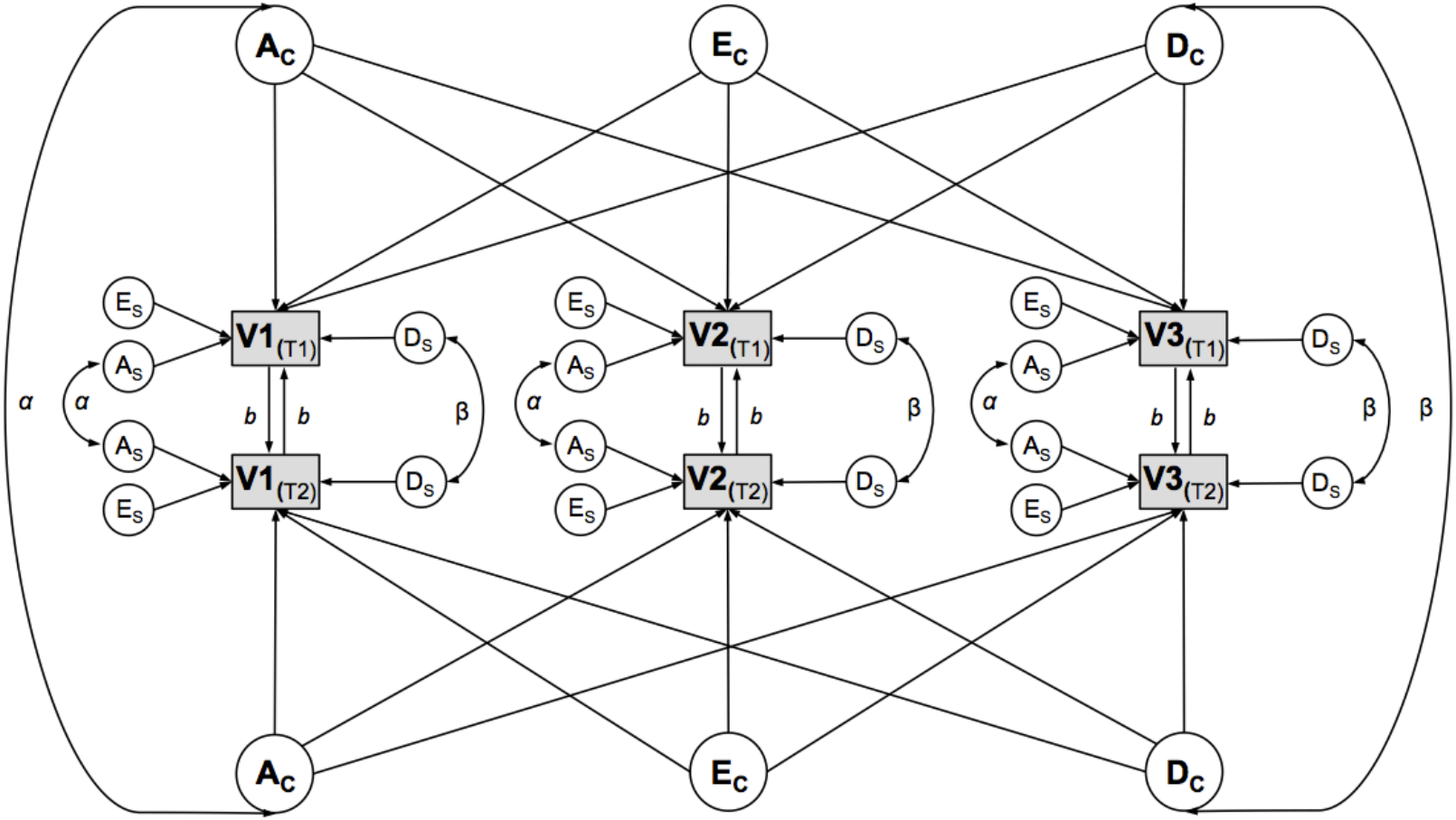
The common pathway model (Figure 2.7) is based on a psychometric model, which assumes that phenotypic covariance is best represented by a single, higher-order latent factor (F) with variance constrained to 1.00. In this model, common genetic/environmental factors (A_C, D_C, E_C) explain a proportion of the variance in the latent factor, which in turn accounts for a proportion of the total variance in each observed phenotype. The remaining variance, unique to each phenotype, is accounted for by specific genetic/environmental factors (A_S, D_S, E_S). Thus, although the independent and common pathway models both incorporate common and specific genetic/environmental factors, they assume that different mechanisms underlie the association between variables.

Figure 2.5 Path diagram for the correlated factors solution of the Cholesky decomposition (trivariate)



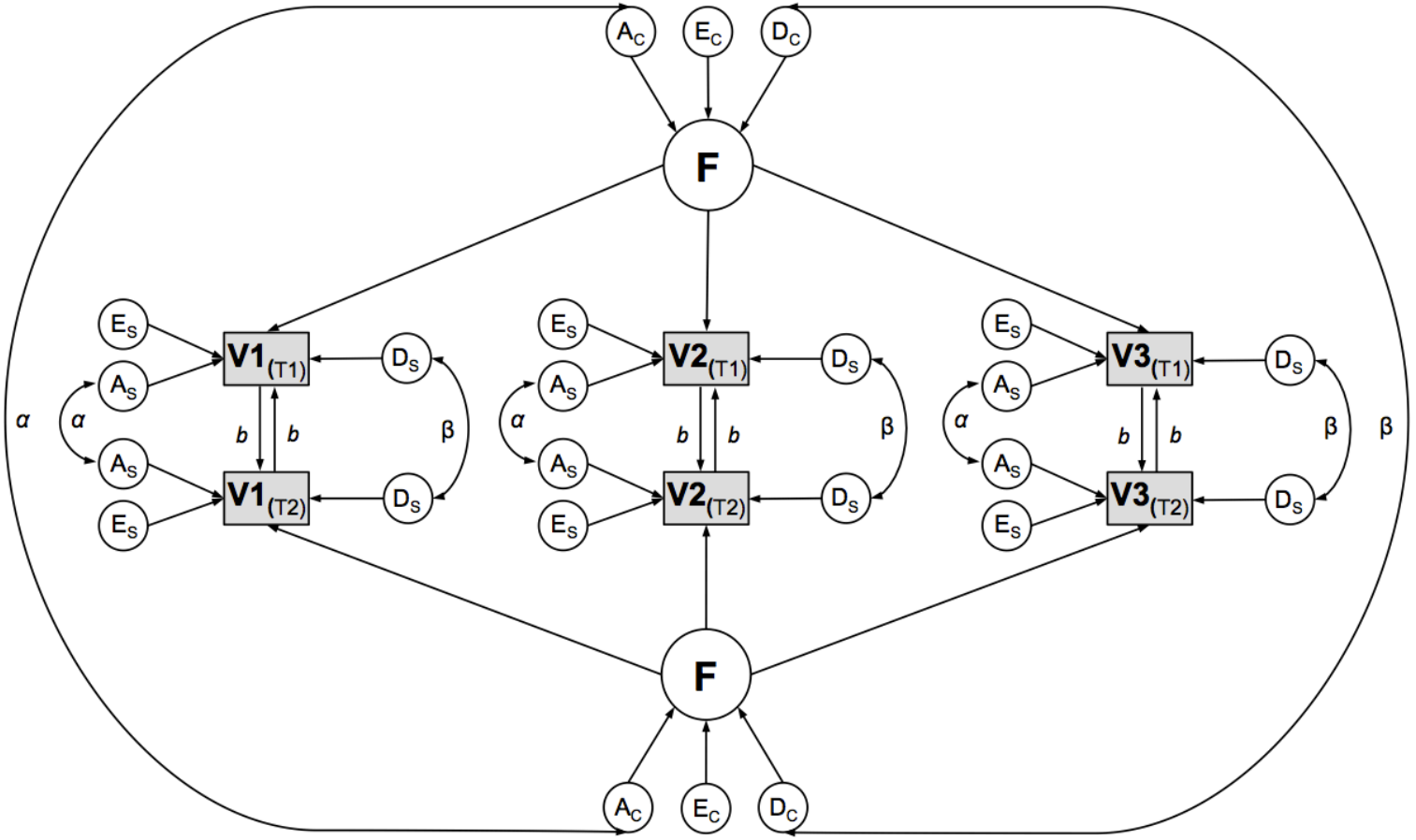
Legend: V1 = variable 1, V2 = variable, V3 = variable 3; T1 = twin 1, T2 = twin 2; *b* = contrast effect; α = additive genetic correlation between T1 and T2, constrained to 1.00 for MZ twins and 0.5 for DZ twins; β = non-additive genetic correlation between T1 and T2, constrained to 1.00 for MZ twins and 0.25 for DZ twins; additive genetic, non-additive genetic and non-shared environmental components presented separately; r_A = additive genetic correlation across phenotypes; r_D = non-additive genetic correlation across phenotypes; r_E = non-shared environmental correlation across phenotypes; all parameter estimates were constrained to be equal for T1 and T2 from a pair.

Figure 2.6 Path diagram for the independent pathway model (trivariate)



Legend: V1 = variable 1, V2 = variable, V3 = variable 3; T1 = twin 1, T2 = twin 2; *b* = contrast effect; α = additive genetic correlation between T1 and T2, constrained to 1.00 for MZ twins and 0.5 for DZ twins; β = non-additive genetic correlation between T1 and T2, constrained to 1.00 for MZ twins and 0.25 for DZ twins; A_C = common additive genetic factor; D_C = common non-additive genetic factor; E_C = common non-shared environmental factor; A_S = specific additive genetic factor; D_S = specific non-additive genetic factor; E_S = specific non-shared environmental factor; all parameter estimates were constrained to be equal for T1 and T2 from a pair.

Figure 2.7 Path diagram for the common pathway model (trivariate)

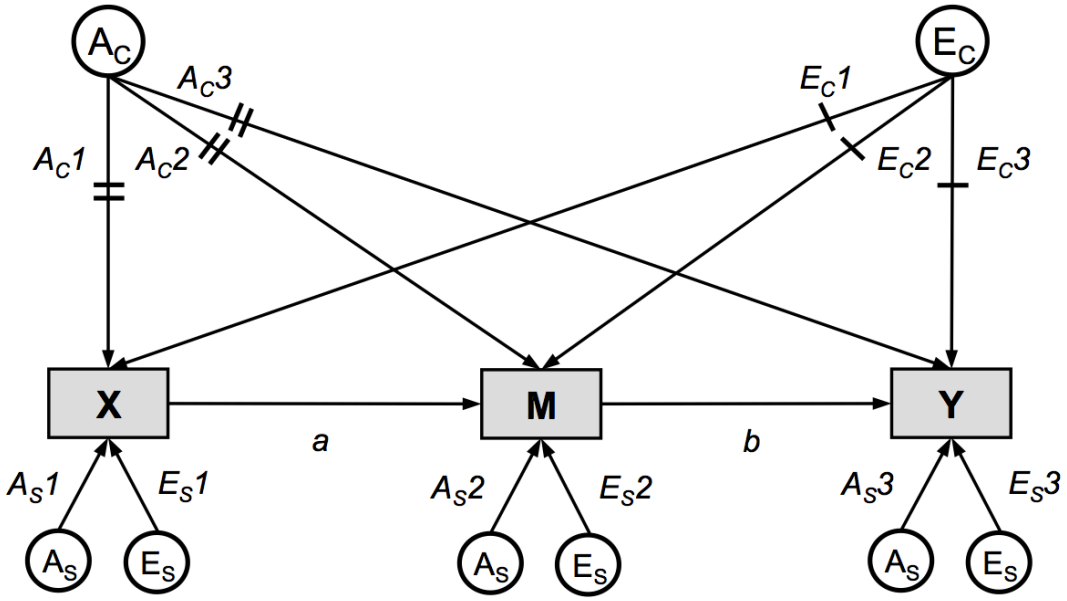


Legend: V1 = variable 1, V2 = variable, V3 = variable 3; T1 = twin 1, T2 = twin 2; *b* = contrast effect; α = additive genetic correlation between T1 and T2, constrained to 1.00 for MZ twins and 0.5 for DZ twins; β = non-additive genetic correlation between T1 and T2, constrained to 1.00 for MZ twins and 0.25 for DZ twins; F = common latent factor; A_C = additive genetic component for F; D_C = non-additive genetic component for F; E_C = non-shared environmental component for F; A_S = specific additive genetic factor; D_S = specific non-additive genetic factor; E_S = specific non-shared environmental factor; all parameter estimates were constrained to be equal for T1 and T2 from a pair.

2.3.7.4 Genetic mediation model

An alternative multivariate model was fit in chapter 6, testing for mediation while also examining genetic/environmental effects, henceforth referred to as the genetic mediation model. It is based on a causal model of personality and depression (Kendler et al., 1993a). In the genetic mediation model, a single, common set of genetic/environmental factors are specified (A_C, E_C), as in the independent pathway model. These factors account for covariation between the observed phenotypes (X, M, Y) and represent a common liability. Causal paths additionally account for a proportion of the variance in M explained by X (path *a*), and a proportion of the variance in Y explained by M (path *c'*). These paths take the form of partial regression coefficients and represent a mediated (indirect) association between X and Y. No direct association between X and Y is specified. The remaining variance in each variable is accounted for by specific genetic/environmental factors (A_C, E_C). To ensure model identification the loading of A_C onto each phenotype is constrained to be equal, as is the loading of E_C. A path diagram is depicted in Figure 2.8.

Figure 2.8 Path diagram for the genetic mediation model



Legend: X = independent variable, M = mediator variable, Y = dependent variable; A_C = common additive genetic factor; E_C = common non-shared environmental factor; A_S = specific additive genetic factor; = specific non-shared environmental factor; a = causal path between X and M; b = causal path between M and Y; all parameter estimates were constrained to be equal for T1 and T2 from a pair; for ease of interpretation, path diagram depicts parameter estimates for one twin only, in line with the original presentation of this model (Kendler and Neale, 1993).

2.3.8 Assumptions of the twin method

The twin method is based on several theoretical assumptions, which if violated impact the quality of research. A strength of the twin method is that these assumptions can be empirically tested, providing sufficient data are available. Key assumptions and their implications are listed below.

2.3.8.1 The equal environments assumption (EEA)

The EEA specifies that the shared environment (C) is no more similar for MZ than DZ twins, or vice versa (Rijsdijk and Sham, 2002). Violations of this assumption can bias the results of twin studies: Greater environmental similarity for MZ than DZ twins will increase MZ twin correlations and inflate estimates of heritability; greater environmental similarity for DZ twins will increase DZ correlations and inflate estimates of C. Perhaps the greatest potential for violations comes from the unequal treatment of twins, with evidence of MZ twins being treated more similarly than DZ twins; however these differences to not appear to unduly bias estimates of genetic/environmental effects for cognitive, emotional and behavioural traits, including symptoms of ADHD (Cronk et al., 2002, Loehlin and Nichols, 1976).

Other studies have examined the effect of zygosity assignment, comparing correctly classified MZ twins to those incorrectly classified as DZ twins. There is some evidence that perceived zygosity assignment biases informant ratings of hyperactive behaviours (i.e. MZ twins misclassified as DZ appear to be treated less similarly that correctly classified MZ twins based on paternal and teacher ratings) (Goodman and Stevenson, 1989b). However, zygosity assignment does not appear to affect levels of parental warmth in the same sample (Goodman and Stevenson, 1991). Other studies have found that self-perceived zygosity does not influence phenotypic similarity for a range of psychiatric traits (Kendler et al., 1993b, Xian et al., 2000). Overall, these results suggest that the EEA generally holds true and that any slight departures should not significantly affect estimates of genetic/environmental effects.

2.3.8.2 Chorionicity

Chorionicity refers to sharing of the chorion, a placental sac that surrounds the embryo during pregnancy. Around two thirds of MZ twins are monochorionic, sharing a single chorion, while the remaining third of MZ twins and all DZ twins are dichorionic (Plomin et al., 2008). Chorionicity is important as it leads to greater similarity of the prenatal environment among monochorionic twins, potentially inflating estimates of heritability. However, it is argued that any such biases are balanced out by the pre/perinatal complications associated with monochorionicity, such as birth defects, low birth weight and in utero competition (Adegbite et al., 2004, Plomin et al., 2008).

2.3.8.3 Gene-environment (GE) interaction

GE interaction refers to a moderating effect of genotype on the environment (Plomin et al., 2008). This is illustrated in studies of differential susceptibility, such as research showing greater rates of depression in response to life stress for carriers of the short (as opposed to long) allele of the serotonin transporter gene (Caspi et al., 2003). In twin research, GE interaction is notoriously difficult to detect without explicit measures of the shared or non-shared environment, meaning that interactions are not modelled under the classical twin design (Rijsdijk and Sham, 2002). Gene by shared environment interaction is therefore subsumed under the variance component A, since MZ twins share 100% of their genes and 100% of their shared environment and will be more similar than DZ pairs. Gene by non-shared environment interaction is subsumed under the component E, since the non-shared environment is unique to individuals and reduces overall twin similarity. Interaction effects can therefore bias heritability estimates up or down.

2.3.8.4 Gene-environment (GE) correlation

GE correlation refers to genetic influences on exposure to environments (Plomin et al., 2008). Active GE correlation occurs when an individual creates environments that are a function of their genotype. A positive correlation will increase estimates of genetic components of variance while a negative

correlation will decrease estimates; however the effect is difficult to identify without longitudinal data and measures of the environment to study effects of mediation (Rijsdijk and Sham, 2002). Passive GE correlation occurs when an individual's environment is determined by their biological relatives, leading to inflated estimates of the shared environment. This effect is difficult to detect using the classical twin design but can be identified via adoption studies (Rijsdijk and Sham, 2002). Evocative GE correlation occurs when individuals are reacted to on the basis of their genetic propensities. An approach for identifying this effect is to examine the correlation between an adoptive environment and a trait in the biological parents of adoptees (Plomin et al., 1977, Plomin et al., 2008).

2.3.8.5 Assortative mating

Assortative mating refers to the non-random pairing of mates on the basis of genetic or environmental factors. The effect can be negative *("opposites attract"*) but is most often positive (*"birds of a feather flock together"*) (Plomin et al., 2008). Positive assortative mating can bias the results of twin studies by reducing estimates of shared environmental effects due to inflated twin correlations for DZ pairs (Rijsdijk and Sham, 2002). However, a useful by-product of assortative mating is increased phenotypic variance within a population. There is some evidence to suggest positive assortative mating for ADHD (van Steijn et al., 2012).

2.5.8.6 Generalisability

It is finally important to consider whether twin samples are representative of non-twin samples. This is because twins show a number of differences from singletons, including reduced birth weight, higher rates of pre-term birth and more perinatal complications (Rijsdijk and Sham, 2002). These do not necessarily lead to phenotypic dissimilarities; for example some research shows that measures of personality are not significantly different in twins versus singletons (Johnson et al., 2002), while other research has identified some twin-singleton differences in psychopathology but not for ADHD (Moilanen et al., 1999). However other research has identified higher levels of ADHD symptoms

in twins when compared to siblings (Levy et al., 1996). Complications associated with twin births, such as low birth weight and prematurity, are also considered risk factors for ADHD (Halmoy et al., 2012, Thapar et al., 2012), although twin research suggests that such perinatal adversity is not necessarily associated with later symptoms of hyperactive behaviour (Goodman and Stevenson, 1989b). The extent to which twin studies are generalisable is therefore not always consistent and is a limitation of the classical twin design.

2.4 POLYGENIC ANALYSES

2.4.1 Profile scoring

The analyses conducted in chapter 7 examine the polygenic basis of ADHD using the profile (allele) score method, consistent with that employed elsewhere (Evans et al., 2009, Hamshere et al., 2013a, Purcell et al., 2009). This method uses two datasets: one to generate a profile score (a discovery set) and a second, independent dataset to test the profile score for association with the phenotype of interest (a target set). The score is generated based on the results of genome-wide association analyses conducted in the discovery set. For each SNP, a reference (risk) allele and its corresponding odds ratio and *p* value from GWAS is identified. A score for each reference allele is generated by computing the log of the odds ratio. The reference alleles and corresponding scores are then used to generate a profile score for each individual in the target set. The profile score is calculated as the number of risk alleles at each SNP multiplied by the log of the odds ratio, with an average score across all non-missing SNPs computed for each individual. An example of the calculation is presented in Box 2.1. To determine which SNPs to include when generating the profile score, different thresholds of *p* value from the initial GWAS can be imposed; for example the profile score might be generated using only SNPs associated with the phenotype at the threshold $p < 0.50$ in the discovery set. Profile scores across different thresholds can be compared. Once generated, profile scores can be tested for association with a phenotype in the target set via regression.

Throughout this thesis, the analysis of genome-wide data and the generation of profile scores was conducted using PLINK version 1.07 (Purcell, 2013, Purcell

et al., 2007). Regressions used to test the profile scores for association with ADHD were conducted using STATA version 10.1 (StataCorp., 2007). The discovery dataset comprised eight samples from the PGC. Profile scores were then generated and tested in two target sets; a proband target set comprising individuals from the IMAGE sample and a population target set comprising individuals from the TEDS and SAIL. Details on data preparation and genomic QC procedures for these samples are described below (section 2.4.2). Further details on the analytic procedures are provided in chapter 7 (section 7.3.2).

Box 2.1 Calculations for genomic profile scores (adapted from Purcell, 2013)

The table below sets out dummy data for four SNPs, which can be used to calculate a profile score for an individual using either standard or dosage format data.

	SNP1	SNP2	SNP3	SNP4
Discovery data				
Allele 1/ Allele 2	A/T	C/G	A/C	C/G
Allele 1 frequency	0.20	0.43	0.02	0.38
Score (log of odds ratio)	1.95	2.04	-0.98	-0.24
Target data - standard format				
Genotype	A/A	G/G	A/C	0/0
No. reference alleles (allele 1)	2	0	1	2*0.38
Target data - dosage format				
Probability allele 1 homozygote	0.98	0.00	0.04	0.41
Probability allele 1 heterozygote	0.02	0.00	0.96	0.46

The upper section of the table gives alleles 1 and 2 for four markers in a discovery dataset. Allele 1 is considered the reference (risk) allele. Allele 1 frequency in the discovery set is then presented in the next row, followed by a score in the final row, which is the log of the odds ratio from GWAS for each reference allele.

The middle section of the table gives the genotype at each marker for a single individual in the target dataset, with data in standard PLINK format. The number of reference alleles carried at each locus is then presented. Note that for SNP4 genotype data were missing. However, the number of risk alleles for missing data points can be imputed as the population frequency of the reference allele multiplied by two (i.e. 2*0.38). The information across SNPs is then used to generate the individual's profile score, calculated as:

*Profile score = ((2*1.95) + (0*2.04) + (1*-0.98) + ((2*0.38)*-0.24)) / 4 = 0.68*

Box 2.1 Continued

The lower section of the table again details the genotype information for a single individual, this time using data in dosage format. Dosage data gives expected, rather than observed, allele counts based on imputed data (see section 2.4.2). Thus, instead of listing the number of alleles carried by an individual, dosage data lists the probability of an individual being homozygous or heterozygous for the risk allele. The probability of an individual being homozygous for the non-reference allele is 1 minus the probability of being homozygous + heterozygous for the reference allele. The profile score is therefore calculated using the homozygote and heterozygote dosages for each SNP:

*Profile score = ((((2*0.98)*1.95)+((1*0.02)*1.95)) + (((2*0)*2.04)+ ((1*0)*2.04))*
*+ (((2*0.04)*-0.98)+((1*0.96)*-0.98)) + (((2*0.41)*-0.24))+((1*0.46)*-0.24))) / 4 = 0.63*

The scoring procedures described above can be implemented in PLINK using the command: *--score*.

2.4.2 Data preparation

The polygenic analyses in chapter 7 used genomic data from the PGC and TEDS. Genomic data were prepared following standard protocol across the respective datasets. This data preparation was conducted by analysts working for the PGC and TEDS and was not conducted as part of this thesis. This approach ensures consistency of the genomic data used in chapter 7 with published and ongoing research from the PGC and TEDS. Details on the data preparation procedures are summarised here.

2.4.2.1 PGC data preparation

This section details the stringent QC pipeline imposed for data preparation in the PGC (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). Details on initial exclusions of individuals (e.g. based on problems with hybridisation, low genotype call rates) can be found for the respective samples by following the key references in Table 2.4.

Raw genotype and phenotype data for each sample were uploaded to a central server to ensure parity of processing. Genotype data were initially pruned to remove SNP missingness (remove SNPs > 5% missing across sample). Data were then pruned to remove individual missingness (remove individuals missing > 2% of genotype) and autosomal heterozygosity deviation, then re-pruned to remove SNP missingness (remove > 2% missing). Data were then pruned for differences in SNP missingness between cases and controls (remove SNPs with differences > 0.02) and for deviations from Hardy-Weinberg equilibrium (HWE; i.e. constancy of genotype and allele frequencies) at the threshold < $1*10^{-6}$ for ADHD cases and < $1*10^{-10}$ for controls. The autosomal SNPs directly genotyped across all platforms (i.e. SNPs common to the different arrays used across PGC samples; see Table 2.3) were then extracted and pruned to remove SNPs in linkage disequilibrium (LD) at the threshold R^2 > 0.05 and SNPs with a minor allele frequency (MAF) < 5%. The resultant set of post-QC SNPs was taken forward for tests of population structure and for imputation.

The PGC datasets included a mixture of population-based samples from ADHD cases and controls (IMAGE 2, China, Germany, Spain, ROI/UK) and family-based samples comprising ADHD probands from trios (CHOP, PUWMA, Canada, IMAGE). Population-based association studies are susceptible to bias introduced by systematic differences in allele frequencies as a result of population structure and ancestry (i.e. population stratification, Benyamin et al., 2009). To control for this, twenty principal components (PCs) were estimated using post-QC SNPs from the five population-based samples, using the programme EIGENSTRAT (Price et al., 2006). PCs represent continuous axes of genetic variation that can be included as covariates in genome-wide analyses to control for stratification effects. Data from family-based samples are exempt from population stratification as they are based on the within-family transmission of alleles from parents to affected offspring. Such data are typically analysed using a transmission disequilibrium test or haplotype relative risk approach; however to enable comparable analyses across the family and population-based samples in the PGC, data from trios were used to generate pseudo-controls (Cordell and Clayton, 2002, Cordell, 2004, Cordell et al., 2004). Pseudo-controls are derived from the untransmitted parental alleles within a family trio: thus, at a single locus, if an ADHD proband had the genotype AC,

with parental genotypes of TA and GC, the pseudo control would be assigned the untransmitted genotype of TG.

Genotype imputation was performed using the pre-phasing/imputation stepwise approach implemented in IMPUTE2 and SHAPEIT (Delaneau et al., 2012, Howie et al., 2012). The reference set for imputation consisted of 2,186 phased haplotypes from the full 1000 Genomes Project dataset, providing information on 40,318,245 markers (1000 Genomes Project, 2013). This large number of markers included SNPs and structural variants with minor allele frequencies of 1% or higher identified through sequencing of the human genome. Imputed markers were excluded when evaluation of the Lambda statistic for genomic control (λ_{GC}) identified control allele frequencies < 0.005 or > 0.995, when imputation quality values were low (< 0.2), or when markers were genotyped only in the smallest sample set. Imputation of the X chromosome was conducted for all subjects passing QC for the autosomal analyses, implemented separately for males and females; however only the autosomal SNPs from chromosomes 1 to 22 were included in the final datasets used in this thesis. Following imputation, approximately 40 million markers were present per PGC sample (see Table 2.4).

2.4.2.2 TEDS data preparation

TEDS data preparation followed a similarly stringent process (Trzaskowski et al., in press). Buccal samples were collected from 3,747 children, of which 3,677 samples successfully hybridised to the genotyping array (see Table 2.4). Individuals were excluded based on low genotype call rate, hybridisation intensity outliers, ancestry outliers, relatedness, sex differences, and low concordance in re-genotyping analyses (conducted to verify the quality of hybridisation to the genotype array). This left a sample of 3,152 individuals, genotyped for 932,533 SNPs. SNP-based pruning was then conducted to remove markers with MAF < 1% and those that deviated from HWE at the threshold < 10^{-20}, leaving a total of 690,943 post-QC SNPs.

TEDS is a population-based sample and thus susceptible to stratification effects. The package EIGENSTRAT (Price et al., 2006) was therefore used to

remove SNPs in high LD (r^2 > 0.2; 105,556 SNPs remaining) and generate eight PCs for inclusion as covariates in analyses; the significance of PCs was confirmed using the Tracey-Widom test (Patterson et al., 2006). Genotype imputation was performed on the post-QC SNP set, using Central European HapMap phase 2 and 3 SNP data as a haploid reference panel (Altshuler et al., 2010, Frazer et al., 2007) and the Wellcome Trust Case/Control Consortium 2 (WTCCC2) control SNP data as a diploid reference panel (Wellcome Trust Case Control Consortium., 2007). Imputation was performed for autosomal SNPs only, using the package IMPUTE2 (Howie et al., 2012), with exclusions made by setting a high threshold for imputation quality (≥ 0.98 for HapMap 2 and 3, ≥ 0.90 for WTCCC2). Following imputation, a total of 1,724,205 SNPs were available for inclusion in analyses.

Table 2.3 SNP arrays and number of imputed SNPs across IMAGE datasets

Sample (key reference)	Genotyping platform	N SNPs post-imputation
CHOP (US) (Elia et al., 2010)	Trios: Illumina Infinium II HumanHap550 BeadChip	40,273,813
PUWMA (US) (Mick et al., 2010)	Trios: Illumina Human 1M BeadChip and Illumina Human 1M-Duo array	40,275,990
IMAGE 2 (DE, NL, ROI, UK, US) (Neale et al., 2010a)	Cases: Affymetrix 5.0 array Controls: Affymetrix 6.0 array	40,258,828
Canada (Lionel et al., 2011)	Trios: Affymetrix 6.0 array	40,280,632
China (Yang et al., 2013)	Cases: Affymetrix 6.0 array Controls: Affymetrix 6.0 array	40,283,324
Germany (Hinney et al., 2011)	Cases: Illumina Human660W-Quad v1 BeadChip Controls: Illumina HumanHap550 v3 array	40,273,813
Spain (Ribasés et al., 2009)	Cases: SNPlex platform Controls: SNPlex platform	40,280,632
ROI/UK (Stergiakouli et al., 2012)	Cases: Illumina Human660W-Quad v1 BeadChip Controls: Illumina Human 1.2M BeadChip	40,273,813
IMAGE (Neale et al., 2008)	Trios: Perlegen 600k array	40,262,315
TEDS (Trzaskowski et al., in press)	General population: Affymetrix 6.0 array	1,724,384

Note: Genotyping platform denotes arrays used across samples, from Illumina (Illumina, San Diego, CA, USA), Affymetrix (Affymetrix, Santa Clara, CA, USA), SNPlex (Applied Biosystems, Foster City, CA, USA), or Perlegen (Perlegen Sciences, Mountain View, CA, USA).

3. THE AETIOLOGICAL OVERLAP BETWEEN PARENT, TEACHER AND SELF-RATINGS OF ADHD SYMPTOMS

3.1 OVERVIEW

The aim of chapter 3 was to examine the aetiological overlap between parent, teacher and self-ratings of ADHD symptoms. Participants were 6,372 early-adolescent twin pairs aged 11-12 years. ADHD symptoms were rated by parents, teachers and children using the Strengths and Difficulties Questionnaire (SDQ) hyperactivity scale. Univariate structural equation modelling estimated broad-sense heritability of 82% for parent ratings, 60% for teacher ratings and 48% for child self-ratings. *Post-hoc* analyses revealed significantly higher heritability for same-teacher than different-teacher ratings of ADHD symptoms (76% vs. 49%). In the multivariate modelling, a common pathway model best explained the relationship between different informant ratings, with common genetic influences accounting for 84% of the covariance between parent ratings, teacher ratings and child self-ratings. This indicates that despite different heritabilities, parent, teacher and self-ratings account for some of the same aspects of ADHD-related behaviours.

3.2 INTRODUCTION

The methods used to assess the symptoms of attention deficit hyperactivity disorder (ADHD) vary throughout the lifespan. In childhood and early adolescence the symptoms are typically rated by parents and teachers; in later adolescence and adulthood the symptoms are more frequently self-rated (Asherson, 2005). Parent, teacher and self-ratings of ADHD symptoms correlate only moderately, around r = 0.3 to 0.5 (Achenbach and Rescorla, 2000, Goodman, 2001, Zucker et al., 2002), indicating that different informants may provide different perspectives on ADHD-related behaviours. Characterising the full extent of the phenotypic and aetiological relationships between self and

other informant ratings is particularly relevant in understanding the developmental course of ADHD, since self-ratings are increasingly relied upon in the transition into adulthood. Furthermore, the success of neurobiological and molecular genetic research into ADHD depends on the quality of ratings.

Univariate twin studies suggest that the heritability estimates for ADHD symptoms are to some extent informant-specific. Parent and teacher ratings of child and adolescent ADHD symptoms typically yield high heritability estimates (70-80%; Nikolas and Burt, 2010). In contrast, studies that use self-ratings consistently estimate lower heritability (<50%). This is true of self-ratings obtained using rating scales or via interviews during adolescence (Ehringer et al., 2006b, Martin et al., 2002, Young et al., 2000), and of retrospective and current self-ratings obtained in adulthood (Boomsma et al., 2010, Haberstick et al., 2008, Kan et al., 2013, Larsson et al., 2012b, Schultz et al., 2006, Van Den Berg et al., 2006). Some studies also estimate lower heritability when different teachers, rather than the same teacher, rate each twin from a pair (Derks et al., 2006, Hartman et al., 2007, Saudino et al., 2005, Simonoff et al., 1998). One explanation for low heritability estimates is low reliability. This leads to the attenuation of monozygotic (MZ) cross-twin within-trait correlations and imposes a ceiling limit on heritability estimates by increasing measurement error (Rijsdijk and Sham, 2002). This has been proposed as an explanation for the lower heritability estimated for different-teacher ratings of ADHD (Hartman et al., 2007) and could similarly account for the lower heritability of self-ratings.

The heritability of parent ratings of ADHD is often broad-sense, indicating non-additive as well as additive genetic influences on behaviour (Burt, 2009). Conversely, the heritability of teacher and self-ratings tends to reflect only additive genetic influences. The genetic non-additivity found for parent ratings could reflect a contrast effect, whereby parents contrast the behaviour of their twins and underestimate the similarity of dizygotic (DZ) twins (Simonoff et al., 1998, Wood et al., 2010b). In genetic modelling, contrast effects and genetic non-additivity both lead to low cross-twin within-trait correlations for DZ twins. Contrast effects can be distinguished from genetic non-additivity by greater variance in the behaviours of DZ than MZ twins (Neale and Maes, 2004).

Because of these nuances, an important question is whether different informants actually rate the same aspects of ADHD-related behaviours? Rater differences can occur due to genuine differences in perspective and/or rater biases (Derks et al., 2006), and can be disentangled via multivariate twin studies that use multiple informant data: Unique genetic influences indicate that different informants rate unique but valid aspects of behaviour; unique environmental influences may reflect rater-specific bias (via the shared environmental component) or measurement error (via the non-shared environmental component); common genetic and environmental influences indicate the extent to which different informants rate the same aspects of behaviour (Hewitt et al., 1992).

Bivariate twin studies have identified common as well as unique genetic influences on parent and teacher ratings, suggesting that the same as well as specific aspects of ADHD-related behaviours are rated by different informants (Derks et al., 2006, Hartman et al., 2007, Martin et al., 2002, McLoughlin et al., 2011, Nadder et al., 2002, Simonoff et al., 1998, Thapar et al., 2000). More recent evidence indicates a genetic association between parent and self-ratings of ADHD symptoms that is persistent across the lifespan (Chang et al., 2013). However there are as yet no studies investigating the simultaneous relationship between parent, teacher and self-ratings of ADHD symptoms.

Therefore, the aim of the present study was to examine parent, teacher and self-ratings of ADHD symptoms obtained concurrently for a population-based sample of early-adolescent twins. Univariate genetic modelling assessed the extent to which different informant ratings yielded different heritability estimates. Consistent with previous research, it was hypothesised that heritability estimates for self-ratings would be lower than for parent or teacher ratings. Multivariate genetic modelling evaluated the extent to which the different informant ratings reflected the same and/or specific views of behaviour. It was hypothesised that multivariate analyses would reveal both common and unique aetiological influences for parent, teacher and self-ratings of ADHD symptoms.

3.3 METHOD

3.3.1 Sample and measures

The sample was from the Twins Early Development Study (TEDS). A total of 12,581 individuals from 6,372 twin pairs were included in analyses. The mean age of participating twins was 11.28 years (sd = 0.70). ADHD symptoms were assessed using the five-item hyperactivity scale of the Strengths and Difficulties Questionnaire (SDQ) (Goodman, 2001), completed by parents, teachers and self-rated by children: parent ratings of ADHD were available for 5,590 pairs (including 2 incomplete pairs); teacher ratings were available for 5,217 pairs (including 1,069 incomplete pairs); self-ratings were available for 5,621 pairs (including 84 incomplete pairs); ratings from all three informants were available for 4,432 pairs (including 939 incomplete pairs). A breakdown of the number of pairs by sex, zygosity and informant is presented in Table 3.1. The sample and measures are described in detail in section 2.2.1.

Table 3.1 Number of participating twin pairs by sex, zygosity and informant

	N pairs					
	All	MZM	MZF	DZM	DZF	DZO
P	5590	908	1116	841	976	1749
T	5217	862	1014	781	923	1637
C	5621	918	1113	845	982	1763

Note: Number of twin pairs (N pairs) with parent (P), teacher (T) and child self-ratings (C) of ADHD symptoms available; All = statistic reported for whole sample; MZM = monozygotic males; MZF = monozygotic females; DZM = dizygotic same-sex males; DZF = dizygotic same-sex females; DZO = dizygotic opposite-sex twins.

3.3.2 Statistical analyses

Preliminary analyses were conducted in Stata version 10.1 (StataCorp., 2007). Structural equation modelling was conducted using Mx (Neale et al., 2006). Prior to modelling, raw data were square-root transformed to correct for non-normal distribution and regressed to correct for the effects of age and sex, a standard twin modelling procedure (McGue and Bouchard Jr, 1984). All transformed/ regressed variables showed approximately normal distributions (in Stata: skewness & kurtosis within range ±1).

Cross-twin within-trait, cross-twin cross-trait and phenotypic correlations were derived using a constrained saturated model (section 2.3.5). Univariate sex-limitation models were then fit to decompose the variance in parent, teacher and self-ratings of ADHD symptoms into genetic and environmental components while testing for aetiological sex differences (section 2.3.6). Based on the pattern of twin correlations, the full sex limitation model parameterised additive genetic (A), non-additive genetic (D), and non-shared environmental (E) components of variance. Models including contrast effects (*b*) were additionally fit when low cross-twin within-trait correlations were observed for DZ pairs in the presence of greater variances for DZ than MZ twins, since this is considered indicative of contrast effects and/or sibling interaction (section 2.3.6). ADE and ADE-*b* models were tested separately, since this provides greater power to detect genetic non-additivity (Rietveld et al., 2003). Sex differences in contrast effects were tested by equating the *b* parameter for males and females and examining the change in model fit.

Multivariate genetic models were used to examine the covariance between parent, teacher and self-ratings. These used cross-twin cross-trait correlations to decompose covariation into genetic and environmental components. Contrast effects were included where appropriate, based on the univariate results. Three classes of model were tested, as described in the chapter 2 (section 2.3.7): the triangular (Cholesky) decomposition, from which the mathematically equivalent correlated factors solution was interpreted (Figure 2.5); the independent pathway model (Figure 2.6); and the common pathway model (Figure 2.7).

3.4 RESULTS

3.4.1 Descriptive statistics

Descriptive statistics are presented in Table 3.1. Tests of mean differences by sex were performed on the raw data, using robust regressions in Stata to control for dependence in the observations from twin pairs (Williams, 2000). Mean ADHD symptom scores were significantly higher for males than females based on ratings from parents (t = 22.24, p < .001), teachers (t = 25.20, p < .001) and self-ratings from children (t = 17.00, p < .001).

Table 3.2 Descriptive statistics for all variables

	Mean (Standard Deviation)					
	All	MZM	MZF	DZM	DZF	DZO
P	2.81 (2.25)	3.36 (2.25)	2.29 (1.96)	3.23 (2.39)	2.50 (2.14)	2.81 (2.31)
T	2.20 (2.48)	2.98 (2.74)	1.48 (1.90)	2.92 (2.76)	1.66 (2.06)	2.20 (2.53)
C	3.52 (2.30)	3.81 (2.37)	3.10 (2.12)	3.89 (2.37)	3.29 (2.24)	3.58 (2.31)

Note: descriptive statistics reported for raw data; P = parent ratings of ADHD symptoms; T = teacher ratings; C = child self-ratings; All = statistics reported for whole sample; MZM = monozygotic males; MZF = monozygotic females; DZM = dizygotic same-sex males; DZF = dizygotic same-sex females; DZO = dizygotic opposite-sex twins.

Sex differences in phenotypic variances were examined using Levene's test, also implemented in Stata. Male variances were significantly greater for parent ratings (F = 205.52, p < .001), teacher ratings (F = 665.24, p < .001) and child self-ratings (F = 59.94, p < .001). Variances were also significantly greater for DZ than MZ twins based on parent ratings (F = 16.95, p < .001) and to a lesser extent teacher ratings (F = 6.50, p < .05), but with no variance differences by zygosity for the child self-ratings (F = 3.43, p = .06). Variance differences were confirmed using the saturated model in Mx, which indicated that phenotypic variances for parent, teacher and child self-ratings could not be constrained to be equal by sex (χ^2 = 214.24, df = 6, p < .001) and that the variance in parent ratings could not be equated across zygosity (χ^2 = 10.02, df = 2, p < .01).

3.4.2 Correlations

Phenotypic correlations (95% confidence intervals) were 0.34 (0.32, 0.36) for parent ratings with teacher ratings, 0.45 (0.45, 0.47) for parent ratings with child self-ratings, and 0.29 (0.27, 0.31) for teacher ratings with child self-ratings. This indicates moderate agreement between the different informants when rating the symptoms of ADHD. Non-overlapping confidence intervals indicated that the correlations were significantly different: the strongest correlation was for parent with child ratings, while the weakest was for teacher with child ratings.

Twin correlations are presented by sex and zygosity in Tables 3.3 and 3.4. For parent ratings, the DZ cross-twin within-trait correlations (Table 3.3) were less than half the MZ correlations. This could be considered indicative of non-additive genetic influences on phenotypic variance, however when interpreted

alongside the significantly greater phenotypic variance for DZ than MZ pairs this correlational pattern suggests the presence of a contrast effect. For teacher ratings, the DZ cross-twin within-trait correlations were roughly half the size of MZ correlations, suggesting predominantly additive genetic influences. For child self-ratings, the DZ cross-twin within-trait correlations were less than half the MZ correlations, suggesting some non-additive genetic influences. Cross-twin cross-trait correlations (Table 3.4) for the DZ pairs were consistently less than half of those for the MZ pairs, suggesting additive and non-additive genetic influences on phenotypic covariance.

3.4.3 Univariate sex-limitation modelling

Full sex-limitation models indicated significant variance (scalar) sex differences for all informant ratings of ADHD symptoms. Based on the pattern of variances and twin correlations, the fit of ADE and ADE-*b* models were compared for parent ratings of ADHD symptoms. The ADE-*b* model provided the better fit (based on the AIC and BIC fit statistics, see section 2.3.4), from which the most parsimonious solution was an AE model with *b* equated for males and females. For the teacher ratings and child self-ratings, only ADE models were fit. The most parsimonious solutions were an AE model for teacher ratings and an ADE model for self-ratings. Univariate model fit statistics are presented as supplementary materials in Appendix A. Parameter estimates for the best-fitting models are presented in Table 3.5. Broad-sense heritability estimates were 82% for parent ratings, 60% for teacher ratings and 48% for child self-ratings.

Table 3.3. Cross-twin within-trait correlations

	MZM	MZF	DZM	DZF	DZO
P	0.75 (0.72. 0.78)	0.77 (0.74, 0.79)	0.23 (0.17, 0.29)	0.32 (0.26, 0.32)	0.25 (0.21, 0.29)
T	0.63 (0.58, 0.67)	0.57 (0.53, 0.57)	0.29 (0.22, 0.36)	0.33 (0.27, 0.39)	0.31 (0.26, 0.35)
C	0.49 (0.44, 0.53)	0.48 (0.44, 0.52)	0.21 (0.15, 0.27)	0.21 (0.15, 0.27)	0.15 (0.11, 0.19)

Note: cross-twin within-trait correlations presented by sex and zygosity; correlations performed on transformed data regressed on age and sex; P = parent ratings of ADHD symptoms; T = teacher ratings; C = child self-ratings; MZM = monozygotic males; MZF = monozygotic females; DZM = dizygotic same-sex males; DZF = dizygotic same-sex females; DZO = dizygotic opposite-sex twins; 95% confidence intervals in parentheses.

Table 3.4. Cross-twin cross-trait correlations

	MZM	MZF	DZM	DZF	DZO
P & T	0.29 (0.28, 0.32)	0.30 (0.28, 0.33)	0.08 (0.03, 0.14)	0.12 (-0.08,0.17)	0.09 (0.06, 0.12)
P & C	0.35 (0.32, 0.38)	0.37 (0.35, 0.40)	0.07 (0.02, 0.12)	0.12 (0.08,0.17)	0.09 (0.06, 0.12)
T & C	0.26 (0.23, 0.30)	0.25 (0.21, 0.28)	0.09 (0.04, 0.14)	0.16 (0.15, 0.20)	0.10 (0.07, 0.14)

Note: cross-twin cross-trait correlations presented by sex and zygosity; correlations performed on transformed data regressed on age and sex; P & T = correlation of parent ratings for twin 1 with teacher ratings for twin 2; P & C = correlation of parent ratings for twin 1 with child self-ratings for twin 2; T & C = correlation of teacher ratings for twin 1 with child self-ratings for twin 2; MZM = monozygotic males; MZF = monozygotic females; DZM = dizygotic same-sex males; DZF = dizygotic same-sex females; DZO = dizygotic opposite-sex twins; 95% confidence intervals in parentheses.

Table 3.5 Standardised parameter estimates for the best-fitting univariate models

	A^2	D^2	E^2	*b*
P	0.82 (0.80, 0.83)	-	0.18 (0.16, 0.20)	-0.04 (-0.05, -0.03)
T	0.60 (0.58, 0.63)	-	0.40 (0.37, 0.42)	-
C	0.28 (0.15, 0.41)	0.20 (0.06, 0.34)	0.52 (0.49, 0.55)	-

Note: Model denotes best-fitting sex limitation model; A^2 = standardised additive genetic variance component; D^2 = standardised non-additive genetic variance component; E^2 = standardised non-shared environmental variance component; *b* = contrast effect; P = parent ratings of ADHD symptoms; T = teacher ratings; C = child self-ratings; 95% confidence intervals in parentheses.

3.4.4 Multivariate modelling

Based on the univariate results all multivariate models included a scalar to account for the variance sex differences observed for all informant ratings of ADHD symptoms. Each model additionally included a contrast effect parameter (*b*) for parent ratings only. The AIC and BIC statistics indicated a strong preference for the common pathway model, from which a restricted model parameterising ADE influences at the common level (A_C, D_C, E_C) and AE at the specific level (A_S, E_S) provided the best fit. Fit statistics for all multivariate models are presented in Table 3.6. Parameter estimates for the best fitting model are presented in Table 3.7 and a path diagram in Figure 3.1.

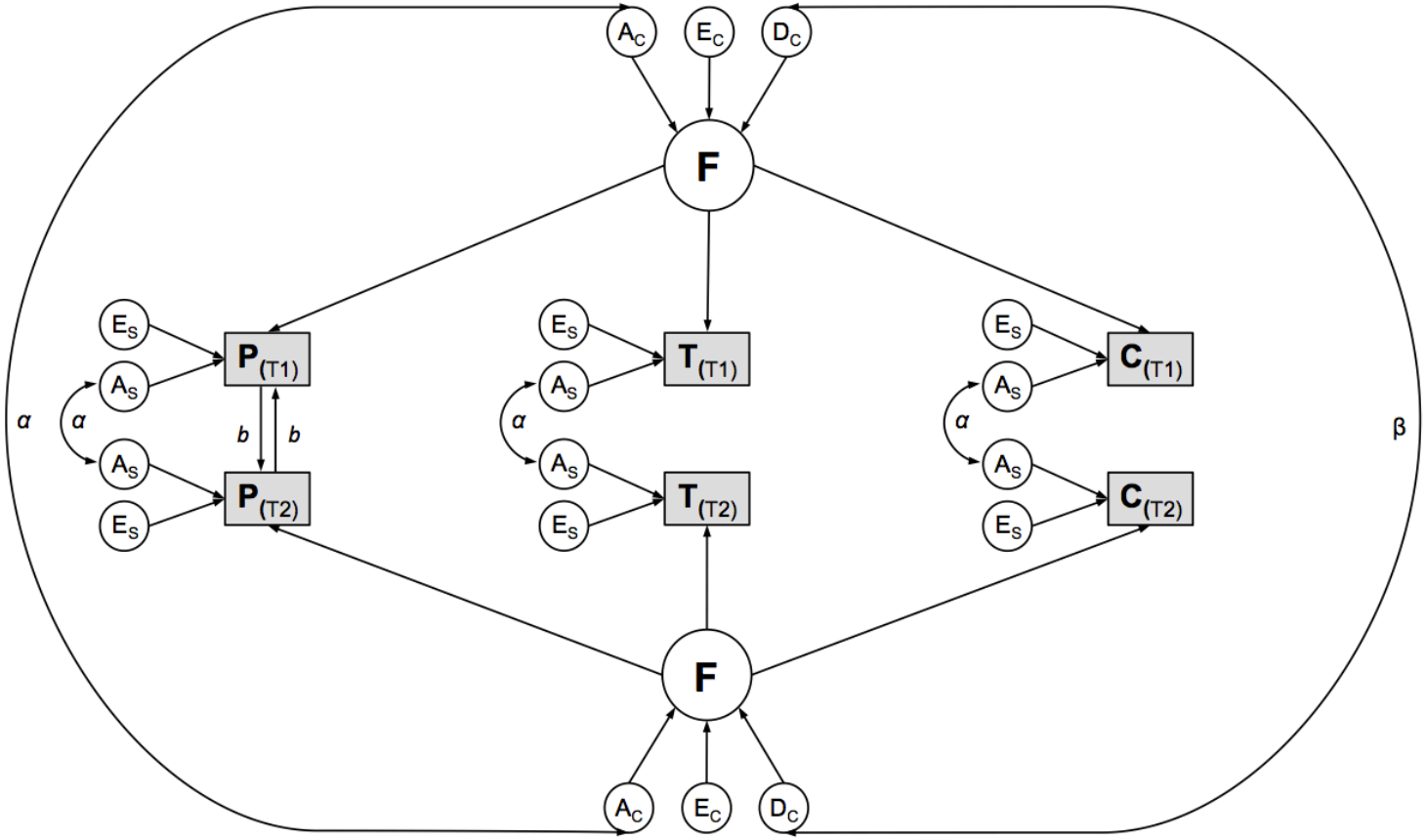
In the best fitting common pathway model, a common latent factor (F) accounted for similarities among the different informant ratings of ADHD symptoms. This factor was highly heritable ($A_C^2+D_C^2 = 0.84$), with the remainder of its variance accounted for by non-shared environmental effects (E_C). The common latent factor accounted for 52% of the total variance in parent ratings, 21% in teacher ratings and 40% in the child self-ratings. This is consistent with the phenotypic correlations in indicating greater agreement between the parent ratings and child self-ratings of ADHD. In turn, genetic influences operating on the common factor accounted for 43% of the total variance in parent ratings, 17% in teacher ratings and 32% in the child self-ratings (see Table 3.8 for percentages and calculations). These results indicate that parent, teacher and child self-ratings assessed some of the same aspects of ADHD-related behaviour, and that common genetic influences accounted for most of the similarity between informants.

Table 3.6 Fit statistics for the multivariate models

Model	Parameters	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated	-	47444.97	31644	-15843.03	-114872.99	-	-	-
CFS	A, D, E, r_A, r_D, r_E, *b*	47499.96	31673	-15846.04	-114972.51	-	-	-
IP	A_C, D_C, E_C, A_S, D_S, E_S, *b*	47500.53	31673	-15845.47	-114972.23	-	-	-
CP	A_C, D_C, E_C, A_S, D_S, E_S, *b*	47509.53	31677	-15844.47	-114985.25	-	-	-
CP	A_C, D_C, E_C, A_S, D_S, E_S	47520.01	31678	-15836.00	-114984.39	10.47	1	<.01
CP	**A_C, D_C, E_C, A_S, E_S, *b***	**47509.54**	**31680**	**-15850.46**	**-114998.38**	**0.01**	**3**	**1.00**
CP	A_C, D_C, E_C, A_S, E_S	47522.28	31681	-15839.72	-114996.39	12.75	4	<.05
CP	A_C, D_C, E_C, E_S, *b*	48723.04	31683	-14642.96	-114404.77	1213.51	6	<.001
CP	A_C, D_C, E_C, E_S	48842.97	31684	-14525.03	-114349.19	1333.43	7	<.001
CP	A_C, E_C, A_S, D_S, E_S, *b*	47530.17	31678	-15825.83	-114979.31	20.64	1	<.001
CP	A_C, E_C, A_S, D_S, E_S	47565.18	31679	-15792.82	-114966.18	55.65	2	<.001
CP	A_C, E_C, A_S, E_S, *b*	47530.96	31681	-15831.04	-114992.05	21.43	4	<.001
CP	A_C, E_C, A_S, E_S	47587.68	31682	-15776.32	-114968.07	78.15	5	<.001
CP	A_C, E_C, E_S, *b*	48887.14	31684	-14480.86	-114327.10	1377.61	7	<.001
CP	A_C, E_C, E_S	48909.48	31685	-14460.52	-114320.31	1399.95	8	<.001

Note: -2LL = log likelihood statistic; DF = degrees of freedom; AIC = Akaike's Information Criterion; BIC = Bayesian Information Criterion; χ^2 = likelihood ratio test (LRT) for difference between full and restricted models; df = difference in degrees of freedom for LRT; *p* = significance of LRT; CFS = correlated factors solution of the Cholesky decomposition; IP = independent pathway model; CP = common pathway model; all models constrained male variances to be a scalar multiple of female variances for parent, teacher and self ratings; contrast effects (*b*) were included for parent ratings only and constrained to be equal for males and females; best-fitting model denoted in **bold**.

Figure 3.1. Path diagram for the best-fitting common pathway model



Legend: path diagram depicts factor loadings onto twin 1 (T1) and twin 2 (T2) for parent ratings (P), teacher ratings (T) and child self-ratings (C) of ADHD symptoms; F = common latent factor; A = additive genetic component of variance; D = non-additive genetic component; E = non-shared environmental component; C suffix denotes common variance component; S suffix denotes specific variance component; *b* = contrast effect; α = coefficient of additive genetic relatedness between T1 & T2, set to 1.00 for MZ pairs and 0.5 for DZ pairs; β = coefficient of non-additive genetic relatedness between T1 & T2, set to 1.00 for MZ pairs and 0.25 for DZ pairs.

The remaining variance for each informant rating of ADHD symptoms was accounted for by specific genetic and environmental factors. The presence of specific genetic influences (A_S) indicated that each informant rated unique but valid aspects of ADHD-related behaviour, whereas the specific non-shared environmental influences (A_S) indicated that the different informant reports were also influenced by the unique environment and/or measurement error.

Table 3.7 Standardised parameter estimates for the best-fitting common pathway model

	F	P	T	C
A_C^2	0.34 (0.13, 0.56)	-	-	-
D_C^2	0.49 (0.28, 0.71)	-	-	-
E_C^2	0.16 (0.14, 0.19)	-	-	-
F^2	-	0.52 (0.48, 0.56)	0.21 (0.19, 0.24)	0.40 (0.36, 0.44)
A_S^2	-	0.36 (0.32, 0.39)	0.43 (0.43, 0.47)	0.16 (0.12, 0.19)
E_S^2	-	0.12 (0.10, 0.14)	0.36 (0.36, 0.49)	0.45 (0.42, 0.45)
b	-	-0.04 (-0.06, -0.02)	-	-

Note: F = latent factor; P = parent ratings; T = teacher ratings; C = child self-ratings; A_C^2 = standardised additive genetic component for latent factor; D_C^2 = standardised non-additive genetic component for latent factor; E_C^2 = standardised non-shared environmental component for latent factor; F^2 = latent factor loading for each phenotype; A_S^2 = specific additive genetic component for each phenotype; E_S^2= specific non-shared environmental component for each phenotype; *b* = contrast effect; 95% confidence intervals in parentheses.

Table 3.8 Percentage of variance due to common vs. specific genetic/ environmental effects

	P	T	C
Common A	18%	7%	13%
Common D	25%	10%	19%
Common E	8%	3%	6%
Specific A	36%	43%	16%
Specific E	12%	36%	45%

Note: percentage of total variance explained in parent ratings (P), teacher ratings (T) and child self-ratings (C), calculated using values in Table 3.7; percentage due to common effects calculated as the standardised common factor loading multiplied by the standardised common parameter estimate, multiplied by 100 (i.e. Common A = [F^2 * A_C^2] * 100); proportion due to specific effects calculated as standardised specific parameter estimate multiplied by 100 (i.e. Specific E = E_S^2 * 100).

3.4.5 *Post-hoc* analyses of same versus different teachers

In the univariate and multivariate genetic modelling, the heritability estimated for teacher ratings was lower than expected based on the results of a recent meta-analysis (Nikolas and Burt, 2010). Previous research indicates that this pattern of results can occur when same and different-teacher ratings of ADHD symptoms are combined (Derks et al., 2006). Accordingly, the sample was stratified based on whether both twins from a pair had either the same teacher (N = 1,868 pairs) or different teachers (N = 3,349 pairs) at school. All genetic analyses were then repeated separately in these groups.

First, univariate sex-limited modelling was repeated. Model fit statistics are presented as supplementary materials in Appendix A. For both the same-teacher and different-teacher groups the most parsimonious models were *AE* scalar models. Parameter estimates for the best-fitting models are presented in Table 3.9. These indicated that the heritability of teacher ratings was higher in the same-teacher group than in the different-teacher group (76% vs. 49%). Non-overlapping confidence intervals for the A^2 parameter estimates indicated that this was a significant difference. A comparison of the heritability estimates derived from parent ratings, teacher ratings (all, same and different) and child self-ratings is presented in Figure 3.2.

Table 3.9 Standardised parameter estimates for same vs. different teacher univariate models

	A^2	E^2
T (same)	0.76 (0.73, 0.78)	0.24 (0.22, 0.27)
T (different)	0.47 (0.42, 0.51)	0.53 (0.49, 0.58)

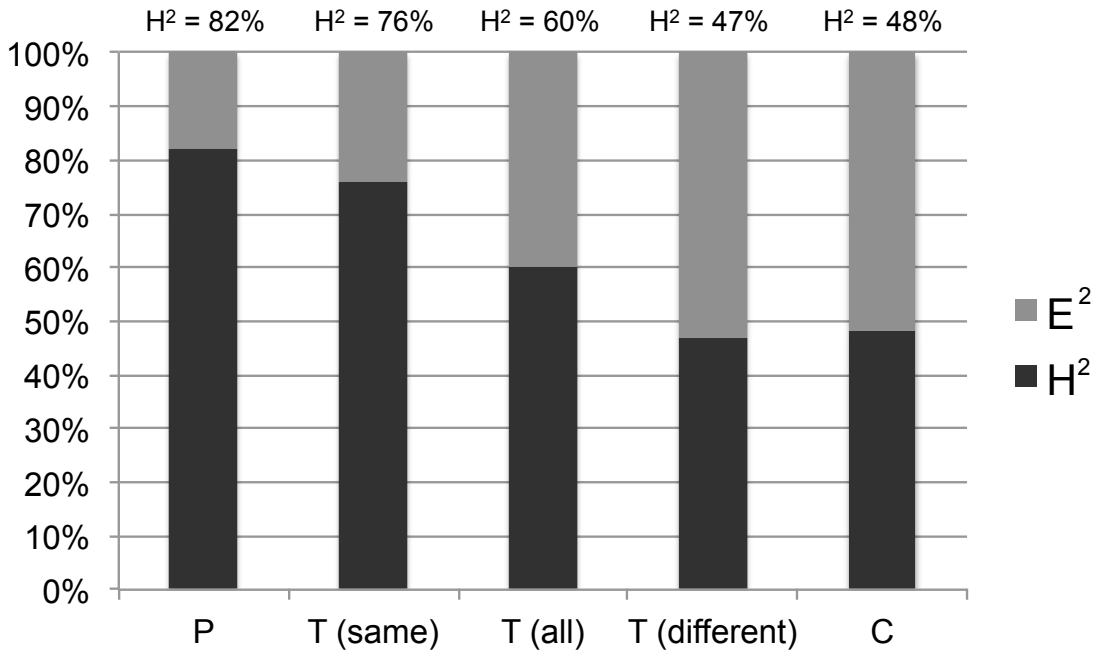
Note: Model denotes best-fitting sex limitation model; A^2 = standardised additive genetic variance component; E^2 = standardised non-shared environmental variance component; *b* = contrast effect; T = teacher ratings for either the same-teacher (same) or different-teacher (different) group; 95% confidence intervals in parentheses.

The common pathway model was then re-fit. For both groups a model that parameterised ADE influences at the common level (A_C, D_C, E_C) and AE at the specific level (A_S, E_S) provided the best fit. The model for the different-teacher group also incorporated a contrast effect (*b*) for parent-rated ADHD symptoms,

however in the same-teacher group the contrast effect for parent ratings was non-significant and could be removed in the interests of model parsimony. The additive genetic variance component for the latent factor (A_C) was also non-significant in the same-teacher group, but was retained in the model since it is considered biologically implausible to find genetic non-additivity in the absence of additive genetic effects (Plomin et al., 2008). Non-significance of these parameter estimates may reflect the smaller sample size of the same-teacher analysis group.

In both the same-teacher and different-teacher models a highly heritable latent factor accounted for covariance among parent, teacher and self-ratings of ADHD symptoms ($A_C^2+D_C^2$ = 0.85 & 0.83 respectively). This is consistent with results reported for the whole sample. Specific genetic influences (A_S^2) for teacher ratings were significantly higher in the same-teacher than different-teacher models, based on non-overlapping confidence intervals. Parameter estimates are presented in Tables 3.10 and 3.11, and model fit statistics in Table 3.12.

Figure 3.2. Broad-sense heritabilities of different informant ratings of ADHD symptoms



Legend: P = parent ratings; T (same) = same-teacher ratings; T (all) = combined same & different teacher ratings; T (different) = different-teacher ratings; C = child self-ratings; H^2 = broad-sense heritability (A^2+D^2); E^2 = non-shared environment.

Table 3.10 Fit statistics for the same-teacher multivariate models

Model	Parameters	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated	-	16592.47	11581	-6569.53	-36483.13	-	-	-
CP	A_C, D_C, E_C, A_S, D_S, E_S, *b*	16633.48	11614	-6594.52	-36590.22	-	-	-
CP	A_C, D_C, E_C, A_S, D_S, E_S	16633.51	11615	-6596.49	-36594.07	0.03	1	0.87
CP	A_C, D_C, E_C, A_S, E_S, *b*	16633.48	11617	-6600.52	-36601.82	0.00	3	1.00
CP	**A_C, D_C, E_C, A_S, E_S**	**16633.51**	**11618**	**-6602.49**	**-36605.67**	**0.03**	**4**	**1.00**
CP	A_C, D_C, E_C, E_S, *b*	17415.20	11620	-5824.80	-36222.56	781.72	6	<.001
CP	A_C, D_C, E_C, E_S	17499.29	11621	-5742.71	-36184.38	865.81	7	<.001
CP	A_C, E_C, A_S, D_S, E_S, *b*	16643.04	11615	-6586.96	-36589.30	9.56	1	<.01
CP	A_C, E_C, A_S, D_S, E_S	16648.58	11616	-6583.42	-36590.40	15.10	2	<.001
CP	A_C, E_C, A_S, E_S, *b*	16643.04	11618	-6592.96	-36600.90	9.56	4	<.05
CP	A_C, E_C, A_S, E_S	16648.68	11619	-6589.32	-36601.95	15.20	5	<.05
CP	A_C, E_C, E_S, *b*	17455.47	11621	-5786.53	-36206.29	821.99	7	<.001
CP	A_C, E_C, E_S	17508.52	11622	-5735.48	-36183.63	875.04	8	<.001

Note: -2LL = log likelihood statistic; DF = degrees of freedom; AIC = Akaike's Information Criterion; BIC = Bayesian Information Criterion; χ^2 = likelihood ratio test (LRT) for difference between full and restricted models; df = difference in degrees of freedom for LRT; *p* = significance of LRT; CP = common pathway model; best-fitting model denoted in **bold**.

Table 3.11 Fit statistics for the different-teacher multivariate models

Model	Parameters	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated	-	30605.70	20006	-9406.30	-67882.65	-	-	-
CP	A_C, D_C, E_C, A_S, D_S, E_S, *b*	30653.03	20039	-9424.97	-67996.20	-	-	-
CP	A_C, D_C, E_C, A_S, D_S, E_S	30664.32	20040	-9415.68	-67994.72	11.29	1	<.01
CP	**A_C, D_C, E_C, A_S, E_S, *b***	**30654.94**	**20042**	**-9429.06**	**-68007.72**	**1.91**	**3**	**0.59**
CP	A_C, D_C, E_C, A_S, E_S	30672.21	20043	-9413.79	-68003.25	19.18	4	<.01
CP	A_C, D_C, E_C, E_S, *b*	31220.09	20045	-8869.92	-67737.63	567.05	6	<.001
CP	A_C, D_C, E_C, E_S	31262.15	20046	-8829.85	-67720.75	609.12	7	<.001
CP	A_C, E_C, A_S, D_S, E_S, *b*	30664.32	20040	-9415.68	-67994.72	11.28	1	<.01
CP	A_C, E_C, A_S, D_S, E_S	30693.19	20041	-9388.81	-67984.44	40.15	2	<.001
CP	A_C, E_C, A_S, E_S, *b*	30668.30	20043	-9417.70	-68005.20	15.27	4	<.01
CP	A_C, E_C, A_S, E_S	30725.78	20044	-9362.22	-67980.62	72.74	5	<.001
CP	A_C, E_C, E_S, *b*	31314.22	20046	-8777.78	-67694.72	661.19	7	<.001
CP	A_C, E_C, E_S	31323.29	20047	-8770.71	-67694.34	670.26	8	<.001

Note: -2LL = log likelihood statistic; DF = degrees of freedom; AIC = Akaike's Information Criterion; BIC = Bayesian Information Criterion; χ^2 = likelihood ratio test (LRT) for difference between full and restricted models; df = difference in degrees of freedom for LRT; *p* = significance of LRT; CP = common pathway model; best-fitting model denoted in **bold**.

Table 3.12 Standardised parameter estimates for the same and different teacher common pathway models

	F	P	T	C
Same				
A_C^2	0.28 (0.00, 0.57)	-	-	-
D_C^2	0.57 (0.28, 0.87)	-	-	-
E_C^2	0.15 (0.11, 0.20)	-	-	-
F^2	-	0.55 (0.49, 0.62)	0.20 (0.17, 0.24)	0.37 (0.32, 0.44)
A_S^2	-	0.30 (0.23, 0.36)	0.58 (0.54, 0.62)	0.18 (0.13, 0.24)
E_S^2	-	0.15 (0.12, 0.18)	0.22 (0.19, 0.25)	0.44 (0.40, 0.48)
Different				
A_C^2	0.34 (0.07, 0.57)	-	-	-
D_C^2	0.49 (0.22, 0.76)	-	-	-
E_C^2	0.17 (0.14, 0.21)	-	-	-
F^2	-	0.50 (0.46, 0.56)	0.22 (0.19, 0.25)	0.40 (0.36, 0.45)
A_S^2	-	0.39 (0.34, 0.44)	0.31 (0.26, 0.35)	0.14 (0.10, 0.19)
E_S^2	-	0.10 (0.08, 0.13)	0.47 (0.43, 0.51)	0.45 (0.42, 0.49)
b	-	-0.05 (-0.08, -0.04)	-	-

Note: upper section gives estimates for same-teacher models; lower section gives estimates for different-teacher models; F = latent factor; P = parent ratings; T = teacher ratings; C = child self-ratings; A_C^2 = standardised additive genetic component for latent factor; D_C^2 = standardised non-additive genetic component for latent factor; E_C^2 = standardised non-shared environmental component for latent factor; F^2 = latent factor loading for each phenotype; A_S^2 = specific additive genetic component for each phenotype; E_S^2= specific non-shared environmental component for each phenotype; *b* = contrast effect; 95% confidence intervals in parentheses.

3.6 DISCUSSION

This study investigated the aetiological relationship between parent ratings, teacher ratings and child self-ratings of ADHD symptoms. There were two main findings. First, heritability estimates were lower for child self-ratings (48%) than for parent (82%) or teacher (60%) ratings, even though all ratings were obtained concurrently during early adolescence. Second, multivariate modelling indicated shared and unique aetiological influences on the different informant ratings, suggesting shared but also rater-specific views of ADHD-related behaviours.

Previous twin studies of self-rated ADHD symptoms have reported univariate heritabilities below 50% in adolescence and adulthood (Boomsma et al., 2010, Ehringer et al., 2006b, Haberstick et al., 2008, Kan et al., 2013, Larsson et al.,

2012b, Martin et al., 2002, Schultz et al., 2006, Van Den Berg et al., 2006, Young et al., 2000). Here, the findings were extended to a younger age group, with a similar heritability estimate (48%) derived when using self-ratings of ADHD symptoms from 11-12 year old twins. This focus on early adolescence indicates that the lower heritability associated with self-ratings is not exclusive to later adolescence or adulthood, and challenges the conclusion that ADHD might be a less heritable phenotype in adults (Boomsma et al., 2010, Saviouk et al., 2011). This is consistent with a recent longitudinal study, which found high heritability of ADHD symptoms from childhood through to adulthood when composite measures of ADHD symptoms were used (Chang et al., 2013).

As expected from a recent meta-analysis (Nikolas and Burt, 2010) the heritability estimate for parent ratings in this study was high (82%), but was lower than expected for teacher ratings (60%). To explore this result, the sample was stratified based on whether the behaviours for both twins from a pair were rated by the same or different teachers and analyses were repeated. The estimate of heritability of teacher ratings was significantly higher in the same-teacher than different teacher group (76% versus 49%). This observation has been reported previously (Derks et al., 2006, Hartman et al., 2007, Saudino et al., 2005, Simonoff et al., 1998) and therefore appears to be a robust finding.

It is noteworthy that the heritability estimates derived from same-teacher ratings were more similar to parent ratings, whereas the estimates from different-teacher ratings were more similar to self-ratings. This suggests that having a single informant rate the behaviours of both twins from a pair (either a parent or the same-teacher) leads to higher heritability estimates than having ratings by different informants for each twin (either the children themselves or different-teachers). There are several possible conclusions.

One conclusion is that the different-informant ratings may be more sensitive to genuine non-shared environmental influences on behaviour, such as peer relationships or teacher characteristics. If this is the case, then different-informant ratings may provide more accurate heritability estimates that better account for non-shared environmental effects. Another conclusion is that of gene-environment interaction, which occurs when genetic influences depend

on the environment. This was supposed in one recent twin study, which suggested that exposure to different teachers and the corresponding classroom environments triggered different externalised behaviours in each twin from a pair (Lamb et al., 2012). A third conclusion is that different-informant ratings are associated with increased measurement error, a likely scenario since reliability between ratings will always be lower when two rather than one rater is involved (unless inter-rater reliability approaches one). If this is the case then the different-informant ratings may underestimate heritability. In the models fit it was not possible to distinguish genuine non-shared environmental effects from error, so it is unclear which of these explanations may be correct.

An additional explanation that must be considered in relation to the low heritability of self-ratings is that children may be unreliable informants when rating their own behaviours. This was the conclusion drawn in one prior twin study that estimated heritability of zero when using child and adolescent self-ratings of ADHD symptoms, also obtained using the SDQ hyperactivity scale (Martin et al., 2002). Previous research indicates that the SDQ hyperactivity scale is less reliable when completed as a self-rating instrument as opposed to being completed by parents or teachers, based on internal consistency and retest stability statistics obtained in childhood and adolescence (Goodman, 2001). Moreover, the internal consistency of self-ratings from the SDQ hyperactivity scale appears to increase with age, from 10-13 years ($\alpha = 0.57$) to 13-16 years ($\alpha = 0.65$) and 16-19 years ($\alpha = 0.66$) (Van Roy et al., 2008). This evidence suggests that children are less reliable informants when rating their own ADHD symptoms, but that the reliability of self-ratings increases across development. In the present study the internal consistency for self-ratings was acceptable ($\alpha = 0.69$), although not as good as for parent ($\alpha = 0.76$) or teacher ($\alpha = 0.86$) ratings. This indicates that the children who participated in this study were reasonably reliable when assessing their own ADHD symptomatology.

In the multivariate genetic modelling a highly heritable latent factor accounted for similarity between parent, teacher and self-ratings of ADHD symptoms, indicating that the overlap between different informant ratings was largely due to a common set of genetic effects. *Post-hoc* analyses showed similar results when same-teacher and different-teacher ratings were considered separately.

However, the loading of teacher ratings onto the latent factor was always significantly lower than the loadings of parent or self-ratings, indicating that the greatest similarity was between parents and children. The weaker association of teacher ratings with this pervasive view is in line with previous studies showing distinct as well as shared aetiological influences for parent and teacher ratings of ADHD symptoms (Derks et al., 2006, Hartman et al., 2007, Martin et al., 2002, McLoughlin et al., 2011, Nadder et al., 2002, Simonoff et al., 1998, Thapar et al., 2000). Because of this, and due to the finding of specific genetic influences on parent, teacher and child self-ratings, rater-specific effects are likely to be valid indicators of different aspects of ADHD-related behaviours, perhaps reflecting differences at home and at school.

Finally, the present analyses provided information on the role of contrast effects and genetic non-additivity across different informant ratings of ADHD. Consistent with previous research using the SDQ hyperactivity scale in this sample, the univariate modelling identified significant contrast effects for parent ratings only (Price et al., 2005, Price et al., 2001, Saudino et al., 2005). Conversely, there were significant non-additive genetic influences on self-ratings, a finding not reported previously. The multivariate model also included non-additive genetic influences on the common factor, indicating that these were important with regard to the overlap between informants. This is particularly interesting owing to the greater power of multivariate models when estimating parameters (Schmitz et al., 1998).

The results should be interpreted in the context of several limitations. First, this study examined ADHD symptoms in a population-based twin sample, meaning that results may not generalise to clinical cases of ADHD. Second, this study used a short, five-item measure of ADHD symptoms (the SDQ hyperactivity scale) rather than an 18-item questionnaire based on DSM-IV. This approach was taken because self-ratings on more comprehensive measures of ADHD symptoms were unavailable. Third, because the SDQ was used, it was not possible to examine the ADHD dimensions of hyperactivity-impulsivity and impulsivity separately and across raters. ADHD is a heterogeneous disorder, and the two dimensions are not perfectly correlated at the phenotypic or genetic level (Greven et al., 2011c, Larsson et al., 2012b, McLoughlin et al.,

2007). Accordingly, one recent twin study found that parents and teachers rated unique aspects of inattentive and hyperactive-impulsive behaviours (McLoughlin et al., 2011).

There are two main implications that arise from the results of this study. First, the identification of a highly heritable common factor suggests that clinical and aetiological investigations of ADHD may benefit from combining data from multiple informants in order to create a pervasive, more heritable phenotype. A multi-rater composite has the effect of reducing measurement error, thereby increasing power for tests of association with genetic, environmental and neurobiological variables. The second implication is for the understanding of self-rating measures that are used in most adult studies of ADHD. The findings in this study suggest that self-ratings in childhood, when used as the sole measure of ADHD symptoms, may underestimate heritability. This means that that future research should collect multiple informant data alongside self-ratings of ADHD symptoms whenever possible. These implications were recently borne-out in a longitudinal twin study that showed high heritability for ADHD symptoms across the lifespan when using a composite measure of parent and self-ratings of ADHD (Chang et al., 2013).

4. AETIOLOGICAL ASSOCIATIONS OF THE ADHD SYMPTOM DIMENSIONS WITH CLONINGER'S DIMENSIONS OF TEMPERAMENT

4.1 OVERVIEW

The aim of chapter 4 was to assess the extent to which ADHD symptoms of hyperactivity-impulsivity and inattention were associated with Cloninger's dimensions of temperament, including investigation of the underlying aetiology of associations observed. Participants were 886 adult twin pairs aged 19-20 years. ADHD symptoms were assessed using DSM-IV based rating scales. Temperament was assessed using the temperament and character inventory for the dimensions of novelty seeking, harm avoidance, reward dependence and persistence. All measures were self-rated. Structural equation modelling revealed a significant genetic correlation of novelty seeking with hyperactivity-impulsivity and inattention, and of harm avoidance and persistence with inattention only. This suggests that unique profiles of temperament are genetically related to the two ADHD symptom domains in adults.

4.2 INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) persists into adulthood in around two-thirds of cases (Faraone et al., 2006a), with adult prevalence estimated at 2.5% (Simon et al., 2009). As in childhood, the adult form of the disorder is characterised by core symptoms of hyperactivity-impulsivity and inattention, leading to significant functional and psychosocial impairments (Asherson, 2005). These symptoms vary continuously throughout the general population and the clinical diagnosis of ADHD is thought to reflect the extreme end of a continuously distributed trait (Chen et al., 2008, Larsson et al., 2012a).

Phenotypic analyses from clinical and epidemiological studies indicate that the core ADHD symptoms are heterogeneous and load onto three factors. These include a general factor consisting of both hyperactive-impulsive and inattentive

symptoms, and two separate factors for hyperactivity-impulsivity and inattention alone (Toplak et al., 2009, Toplak et al., 2012). Quantitative genetic studies confirm the heterogeneous expression of ADHD, indicating that the two symptom dimensions are strongly but not perfectly correlated in children, adolescents and adults with genetic correlations of around 0.6 (Greven et al., 2011c, Larsson et al., 2012b, McLoughlin et al., 2007). This heterogeneity is important, since co-occurring behavioural and cognitive phenotypes are noted to differ in their aetiological associations with hyperactivity-impulsivity and inattention (Greven et al., 2011b, Kuntsi et al., in 2013, Wood et al., 2009a). Understanding the sources of heterogeneity has the potential to improve classification of ADHD symptoms and associated comorbidities, and may help to identify homogenous ADHD subtypes for molecular genetic research.

It has previously been argued that the heterogeneous expression of attention deficit hyperactivity disorder (ADHD) can be understood as a consequence of individual differences in temperament (Nigg et al., 2004b). According to Cloninger's psychobiological theory of personality, temperament emerges in early infancy and remains relatively stable throughout later life (Cloninger et al., 1993). This theory further posits that temperament is determined by neurobiological and genetic factors and divided into four dimensions: novelty seeking, harm avoidance, reward dependence and persistence. Adult twin studies converge to suggest that these dimensions are moderately heritable, around 30-50% (Ando et al., 2002, Ando et al., 2004, Gillespie et al., 2003, Heath et al., 1994, Keller et al., 2005, Stallings et al., 1996). This is lower than the heritability estimated in child and adolescent studies based on parent and teacher ratings of ADHD (~70%; Nikolas and Burt, 2010), but is line with heritability estimates obtained from adult studies that use self-ratings (~30%, Boomsma et al., 2010, Larsson et al., 2012).

Clinical studies consistently suggest that adults with ADHD score higher in novelty seeking and harm avoidance than do controls (Anckarsäter et al., 2006, Downey et al., 1997, Faraone et al., 2009, Jacob et al., 2007, Lynn et al., 2005, Müller et al., 2010, Salgado et al., 2009, Smalley et al., 2009). Results relating to reward dependence and persistence are less clear-cut. Some studies have additionally investigated the differential association of temperament dimensions

with the ADHD symptom domains. Lynn et al. (2005) found that novelty seeking was predictive of higher hyperactive-impulsive and inattentive symptom scores, while Faraone et al. (2009) found that hyperactivity-impulsivity and inattention correlated positively with novelty seeking and harm avoidance but negatively with reward dependence and persistence. Salgado et al. (2009) found positive associations of inattention with harm avoidance only, and of hyperactivity-impulsivity with novelty seeking and persistence. However a limitation of these clinical studies is that they may be subject to referral biases that distort the observed phenotypic relationships. The only population-based study of adult ADHD symptoms and Cloninger's temperament to date reported positive associations of total ADHD symptoms with novelty seeking and harm avoidance, of inattentive symptoms with harm avoidance, and of hyperactive-impulsive symptoms with persistence (Gomez et al., 2012). Cloninger's temperament dimensions therefore appear to differ in their relations with the two ADHD domains.

To date, few twin studies have examined the aetiology of the association between ADHD symptoms and temperament. One study found a strong genetic correlation between hyperactivity-impulsivity and novelty seeking, but focused on children and adolescents only and did not address the question of association between novelty seeking and inattention (Wood et al., 2011a). Two other studies have demonstrated a link between ADHD symptoms and novelty seeking in adolescence, suggesting that the two traits contribute to a highly heritable latent phenotype (Young et al., 2009, Young et al., 2000). One of these studies additionally showed that when modelled separately, hyperactivity-impulsivity and inattention symptoms were similarly associated with novelty seeking (Young et al, 2009). However, twin studies have not yet examined ADHD symptoms in relation to harm avoidance, reward dependence and persistence; nor have they examined the relationship between ADHD symptoms and temperament in adults.

The aim of the present study was to examine the associations between ADHD symptoms and Cloninger's temperament dimensions in a population-based sample of adult twins. Understanding these associations may help to identify genetically homogeneous ADHD subtypes based on individual differences in

temperament, and will allow the relationship between temperament and ADHD in adults to be explored. ADHD symptoms of hyperactivity-impulsivity and inattention were examined separately, to determine whether there were differential associations with the four dimensions of temperament. There were three main hypotheses. First, it was hypothesised that there would be positive phenotypic correlations of inattentive symptoms with harm avoidance, of hyperactive-impulsive symptoms with persistence and of both sets of ADHD symptoms with novelty seeking, in line with previous research. Second, for univariate genetic modelling it was hypothesised that heritability estimates would be in the region of 30-50% for all self-reported measures. Third, for the multivariate genetic modelling it was hypothesised that shared genetic influences would account for most of the phenotypic correlations observed.

4.3 METHOD

4.3.1 Sample and measures

The sample was from the Swedish Twin study of Child and Adolescent Development (TCHAD). A total of 1,634 individuals from 868 twin pairs were included in statistical analyses: 140 monozygotic male (MZM), 214 monozygotic female (MZF), 83 dizygotic male (DZM), 145 dizygotic female (DZF) and 286 dizygotic opposite-sex (DZO) pairs. The mean age of participating twins was 19.66 years (SD = 0.46). ADHD symptoms of hyperactivity-impulsivity and inattention were assessed using an 18-item DSM-IV based scale. Temperament dimensions of novelty seeking, harm avoidance, reward dependence and persistence were assessed using a short version of the Temperament and Character Inventory (Cloninger et al., 1993). All measures were self-rated. The sample and measures are described in detail in chapter 2 (section 2.2.2).

4.3.2 Statistical analyses

Preliminary analyses were conducted in Stata version 10.1 (StataCorp., 2007). Structural equation modelling was conducted using Mx (Neale et al., 2006). Prior to modelling, all variables were regressed to control for the effects of age and sex in accordance with standard twin modelling procedures (section 2.3.4).

The ADHD variables were square-root transformed before regression to improve normality of the data distribution (in Stata: skewness=0±1, kurtosis=3±1). Scores for all temperament dimensions were already normally distributed, with the exception of persistence, which was platykurtic (kurtosis = -1.15). Transformation did not normalise the kurtosis of persistence scores. Untransformed scores for all temperament dimensions were therefore included in structural equation modelling.

Cross-twin within-trait, cross-twin cross-trait and phenotypic correlations, derived from a constrained saturated model, were initially examined to provide a preliminary view of the data and to inform genetic analyses (section 2.3.5). Univariate sex-limitation models were then fit to decompose the variance of each phenotype into genetic and environmental components while testing for aetiological sex differences (see section 2.3.6). Based on the pattern of twin correlations, the full sex limitation model parameterised additive genetic (A), shared environmental (C) or non-additive genetic (D), and non-shared environmental (E) components of variance.

A triangular (Cholesky) decomposition was used to examine the extent to which genetic and environmental influences were shared across phenotypes (Figure 2.5, section 2.3.7). Due to the number of variables included in analyses (six), and because the order of variables was arbitrary, the correlated factors solution of the Cholesky decomposition was interpreted (Loehlin, 1996). However, a scalar was included to account for variance sex differences in harm avoidance based on the univariate results. Bivariate heritabilities were calculated to estimate the proportions of the pairwise phenotypic correlations that were due to genetic and environmental influences. Finally, to determine the extent to which novelty seeking was differentially associated with symptoms of hyperactivity-impulsivity and inattention, *post-hoc* analyses were conducted, in which the fit of a trivariate correlated factors solution was compared to that of independent and common pathway models (Figures 2.5 to 2.7, section 2.3.7).

4.4 RESULTS

4.4.1 Descriptive statistics

Descriptive statistics are presented in Table 4.1. To test for mean differences across sex, robust regression analyses were implemented in Stata that controlled for dependence among the data from twin pairs (Williams, 2000). Males scored significantly lower than females for hyperactivity-impulsivity (t = 4.42, p < .001) but not inattention (t = 0.97, p = 0.43). For the temperament dimensions, males scored significantly lower for harm avoidance (t = 12.42, p < .001) and reward dependence (t = 10.04, p < .001), but not for novelty seeking (t = 0.97, p = .334) or persistence (t = -0.34, p = 0.73).

Table 4.1 Descriptive statistics for all variables

	Mean (Standard Deviation)					
	All	MZM	MZF	DZM	DZF	DZO
HI	2.33 (2.12)	1.92 (1.94)	2.22 (2.13)	1.90 (2.00)	2.54 (2.16)	2.66 (2.18)
IA	2.57 (2.37)	2.14 (2.10)	2.42 (2.33)	2.50 (2.49)	2.86 (2.40)	2.90 (2.45)
NS	10.62 (3.52)	10.79 (3.48)	10.02 (3.32)	10.44 (3.70)	10.59 (3.67)	11.10 (3.48)
HA	8.18 (4.62)	6.18 (4.10)	9.42 (4.51)	6.40 (3.98)	9.78 (4.58)	7.95 (4.56)
RD	9.52 (2.59)	8.81 (2.45)	10.15 (2.32)	8.58 (2.59)	9.94 (2.57)	9.49 (2.69)
PS	2.44 (1.57)	2.63 (1.58)	2.42 (1.54)	2.38 (1.59)	2.43 (1.52)	2.38 (1.62)

Note: descriptive statistics based on raw data; HI = hyperactivity-impulsivity; IA = inattention; NS = novelty seeking; HA = harm avoidance; RD = reward dependence; PS = persistence All = statistics reported for whole sample; MZM = monozygotic males; MZF = monozygotic females; DZM = dizygotic same-sex males; DZF = dizygotic same-sex females; DZO = dizygotic opposite-sex twins.

Sex differences in the phenotypic variances were examined using Levene's test for equality of variances, also implemented in Stata. Variances were unequal for hyperactivity-impulsivity (F = 8.17, p < .01), harm avoidance (F = 16.53, p < .001) and persistence (F = 4.55, p < .05), where female variances were significantly greater than the male variances. These results were suggestive of variance sex differences that warranted investigation using sex-limitation models. There were no sex differences in the variances for inattention (F = 0.00, p = 0.98), novelty seeking (F = 0.29, p = 0.59) or reward dependence (F = 0.29, p = 0.59).

Phenotypic variances were also significantly higher for DZ than MZ twins for hyperactivity-impulsivity (F = 3.62, p < .05) and inattention (F = 4.42, p < .05); however there were no significant variance differences by zygosity (p > .05, respectively). Only the variance difference by sex for harm avoidance could be confirmed using a constrained saturated model (χ^2 = 11.51, df = 2, p < .01).

4.4.2 Correlations

Phenotypic correlations are presented in Table 4.2. There was a significant, moderate correlation between hyperactivity-impulsivity and inattention (r = 0.49). There were also modest correlations of novelty seeking with hyperactivity-impulsivity and inattention (r = 0.28 & 0.23, respectively). In contrast, harm avoidance was only correlated with inattention (r = 0.31). Persistence correlated negatively with inattention (r = -0.13) and positively with HI (r = 0.12), indicating a weak but differential association with the two ADHD domains. RD was weakly correlated with IA and HI (r = -0.07 for both). Correlations among the temperament dimensions were weak to modest.

Cross-twin within-trait correlations (Table 4.3) were greater for MZ than DZ twin pairs for most variables, indicating likely genetic contributions to phenotypic variance. However, for hyperactivity-impulsivity the correlations for DZF pairs were the same as those for MZF pairs, indicating possible shared environmental influences. In contrast, the MZ correlations were greater than half the DZ correlations for most temperament dimensions, in particular novelty seeking, suggesting non-additive as well as additive genetic (A) influences. The role of non-additive genetic (D) influences versus shared-environmental (C) influences was therefore investigated fully in univariate modelling. Cross-twin cross-trait correlations (Table 4.4) were suggestive of additive genetic and/or shared environmental influences for the covariance between hyperactivity-impulsivity and inattention. For the associations between ADHD symptoms and the temperament dimensions, the pattern of correlations suggested primarily additive and/or non-additive genetic sources of covariance.

4.4.3 Univariate sex-limited modelling

For each phenotype the fit of full sex-limitation ACE and ADE models was compared to determine relative influences of the C and D, while also testing for aetiological sex differences. Model fit statistics are presented as supplementary materials in Appendix B. For all phenotypes apart from harm avoidance, models specifying qualitative, quantitative and variance sex differences could be rejected in favour of null models that specified no sex differences. For harm avoidance there was evidence of significant variance sex differences, reflecting greater phenotypic variance among females. Comparing across ACE and ADE models, there were significant D influences for novelty seeking and no significant A influences: A was not dropped from this model as it is considered biologically implausible to find genetic dominance in the absence of genetic additivity (Plomin et al., 2008). For all other variables, AE models provided the best fit, with no significant D or C influences. Parameter estimates for the best-fitting models are presented in Table 4.5.

Table 4.2. Phenotypic correlations

	HI	IA	NS	HA	RD
IA	0.49 (0.46, 0.53)	-	-	-	-
NS	0.28 (0.24, 0.33)	0.23 (0.19, 0.28)	-	-	-
HA	0.02 (-0.03, 0.07)	0.31 (0.26, 0.35)	-0.22 (-0.27, -0.17)	-	-
RD	-0.07 (-0.12, -0.01)	-0.07 (-0.13, -0.02)	-0.02 (-0.07, 0.03)	0.03 (-0.02, 0.09)	-
PS	0.12 (0.07, 0.17)	-0.13 (-0.18, -0.08)	-0.17 (-0.22, -0.12)	-0.18 (-0.23, -0.13)	0.03 (-0.02, 0.08)

Note: phenotypic correlations were equated for all sex and zygosity groups, using a constrained Gaussian decomposition fit in Mx; correlations performed on data regressed on age and sex, with ADHD variables transformed to normality; HI = hyperactivity-impulsivity; IA = inattention; NS = novelty seeking; HA = harm avoidance; RD = reward dependence; PS = persistence; 95% confidence intervals in parentheses.

Table 4.3. Cross-twin within-trait correlations

	MZM	MZF	DZM	DZF	DZO
HI	0.38 (0.21. 0.53)	0.35 (0.23, 0.46)	0.23 (0.00, 0.43)	0.33 (0.16, 0.48)	0.07 (-0.07, 0.21)
IA	0.32 (0.16, 0.46)	0.41 (0.30, 0.52)	0.22 (-0.04, 0.43)	0.14 (-0.05, 0.31)	0.17 (0.03, 0.31)
NS	0.46 (0.30. 0.58)	0.47 (0.36, 0.57)	0.05 (-0.19 0.28)	0.01 (-0.16, 0.19)	0.08 (-0.06, 0.22)
HA	0.43 (0.28. 0.56)	0.51 (0.40, 060)	0.10 (-0.15, 0.33)	0.19 (0.02, 0.35)	0.18 (0.03, 0.31)
RD	0.44 (0.28. 0.58)	0.39 (0.25, 0.50)	0.00 (-0.24, 0.23)	0.12 (-0.06, 0.30)	0.09 (-0.06, 0.23)
PS	0.31 (0.13, 0.47)	0.33 (0.19, 0.45)	0.00 (-0.25, 0.24)	0.20 (0.02, 0.36)	0.24 (0.09, 0.37)

Note: cross-twin within-trait correlations presented by sex and zygosity; correlations performed on data regressed on age and sex, with ADHD variables transformed to normality; HI = hyperactivity-impulsivity; IA = inattention; NS = novelty seeking; HA = harm avoidance; RD = reward dependence; PS = persistence; MZM = monozygotic males; MZF = monozygotic females; DZM = dizygotic same-sex males; DZF = dizygotic same-sex females; DZO = dizygotic opposite-sex twins; 95% confidence intervals in parentheses.

Table 4.4. Cross-twin cross-trait correlations

	MZM	MZF	DZM	DZF	DZO
HI & IA	0.29 (0.17. 0.40)	0.27 (0.18, 0.35)	0.26 (0.08, 0.42)	0.19 (0.04, 0.32)	0.07 (-0.04, 0.18)
HI & NS	0.21 (0.09. 0.31)	0.18 (0.09, 0.26)	-0.08 (-0.25, 0.08)	0.14 (0.02, 0.26)	-0.03 (-0.13, 0.07)
HI & HA	-0.02 (-0.13, 0.09)	-0.01 (-0.09, 0.07)	0.18 (0.02, 0.34)	0.06 (-0.07, 0.18)	0.08 (-0.02, 0.18)
HI & RD	-0.06 (-0.187, 0.06)	0.07 (-0.02, 0.16)	0.07 (-0.10, 0.23)	-0.03 (-0.15, 0.10)	-0.18 (-0.28, -0.08)
HI & PS	0.01 (-0.11, 0.13)	0.07 (-0.02, 0.16)	-0.01 (-0.17, 0.15)	0.02 (-0.10, 0.14)	0.04 (-0.07, 0.14)
IA & NS	0.21 (0.10, 0.32)	0.26 (0.18, 0.33)	0.01 (-0.16, 0.18)	0.10 (-0.03, 0.23)	-0.03 (-0.13, 0.07)
IA & HA	0.10 (-0.02, 0.20)	0.15 (0.07, 0.23)	0.06 (-0.13, 0.24)	0.14 (0.01, 0.27)	0.10 (0.00, 0.21)
IA & RD	-0.03 (-0.14, 0.09)	0.02 (-0.07, 0.11)	0.06 (-0.11, 0.24)	0.06 (-0.07, 0.19)	-0.07 (-0.17, 0.04)
IA & PS	-0.06 (-0.18, 0.06)	-0.12 (-0.21, -0.03)	0.06 (-0.11, 0.23)	-0.08 (-0.20, 0.05)	-0.08 (-0.18, 0.02)
NS & HA	-0.14 (-0.24, -0.03)	-0.15 (-0.23, -0.07)	-0.09 (-0.26, 0.08)	0.01 (-0.11, 0.14)	-0.02 (-0.12, 0.08)
NS & RD	-0.05 (-0.16 0.05)	-0.06 (-0.14, 0.03)	0.14 (-0.04, 0.30)	-0.03 (-0.16, 0.10)	-0.04 (-0.14, 0.06)
NS & PS	0.13 (0.03, 0.24)	-0.09 (-0.17, 0.00)	0.02 (-0.15, 0.20)	0.02 (-0.10, 0.15)	-0.06 (-0.16, 0.04)
HA & RD	-0.18 (-0.28, -0.06)	0.02 (-0.07, 0.10)	-0.13 (-0.30, 0.04)	0.01 (-0.12, 0.13)	-0.01 (-0.11, 0.10)
HA & PS	-0.09 (-0.20, 0.02)	-0.09 (-0.18, -0.01)	-0.01 (-0.18, 0.15)	-0.15 (-0.27, -0.02)	-0.06 (-0.16, 0.05)
RD & PS	-0.02 (-0.13, 0.10)	0.09 (0.00, 0.18)	-0.04 (-0.21, 0.14)	0.10 (-0.03, 0.23)	-0.02 (-0.12, 0.09)

Note: cross-twin cross-trait (CTCT) correlations presented by sex and zygosity; correlations performed on data regressed on age and sex, with ADHD variables transformed to normality; HI = hyperactivity-impulsivity, IA = inattention, NS = novelty seeking, HA = harm avoidance, RD = reward dependence, PS = persistence; MZM = monozygotic males; MZF = monozygotic females; DZM = dizygotic same-sex males; DZF = dizygotic same-sex females; DZO = dizygotic opposite-sex twins; 95% confidence intervals in parentheses.

Table 4.5 Standardised parameter estimates for the best-fitting univariate models

	Model	A^2	D^2	E^2
HI	No sex difs.	0.38 (0.29, 0.46)	-	0.62 (0.54, 0.71)
IA	No sex difs.	0.40 (0.31, 0.48)	-	0.60 (0.52, 0.69)
NS	No sex difs.	0.00 (0.00, 0.20)	0.46 (0.23, 0.54)	0.54 (0.46, 0.64)
HA	Variance sex difs.	0.45 (0.37, 0.53)	-	0.55 (0.47, 0.63)
RD	No sex difs.	0.37 (0.26, 0.46)	-	0.63 (0.54, 0.74)
PS	No sex difs.	0.34 (0.25, 0.43)	-	0.66 (0.57, 0.75)

Note: Model denotes best-fitting sex limitation model; A^2 = standardised additive genetic variance component; D^2 = standardised non-additive genetic variance component; E^2 = standardised non-shared environmental variance component; HI = hyperactivity-impulsivity; IA = inattention; NS = novelty seeking; HA =harm avoidance; RD = reward dependence; PS = persistence; 95% confidence intervals in parentheses.

4.4.4 Multivariate modelling

Based on the univariate results, the Cholesky decomposition parameterised the variance components ADE. For harm avoidance, male variances were constrained to be a scalar multiple of female variances. The full model was compared to a restricted model that dropped D, which did not result in a significant deterioration in fit (Table 4.6). Parameter estimates are therefore presented for the best-fitting AE model (Table 4.7), depicted in Figure 4.1.

Across phenotypes there was a strong genetic correlation (r_G) between hyperactivity-impulsivity and inattention (0.77), indicating substantial shared genetic influences. Genetic correlations were also significant for inattention with novelty seeking (0.55), harm avoidance (0.34) and persistence (-0.29), and for hyperactivity-impulsivity with novelty seeking (0.45). Among the temperament dimensions there were significant genetic correlations of novelty seeking with harm avoidance (-0.28) and persistence (-0.30), and of harm avoidance with persistence (-0.26). Non-shared environmental correlations (r_E) were weak-to-modest. Of note were the significant correlations between hyperactivity-impulsivity and inattention (0.32), between hyperactivity-impulsivity and novelty seeking (0.19), and between inattention and harm avoidance (0.28).

Table 4.6 Fit statistics for the multivariate models

Model	Parameters	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated	-	36070.26	9460	17150.26	-14479.83	-	-	-
CFS	ADE	36200.85	9552	17096.85	-14730.75	-	-	-
	AE	36229.82	9573	17083.82	-14788.44	28.98	21	0.12

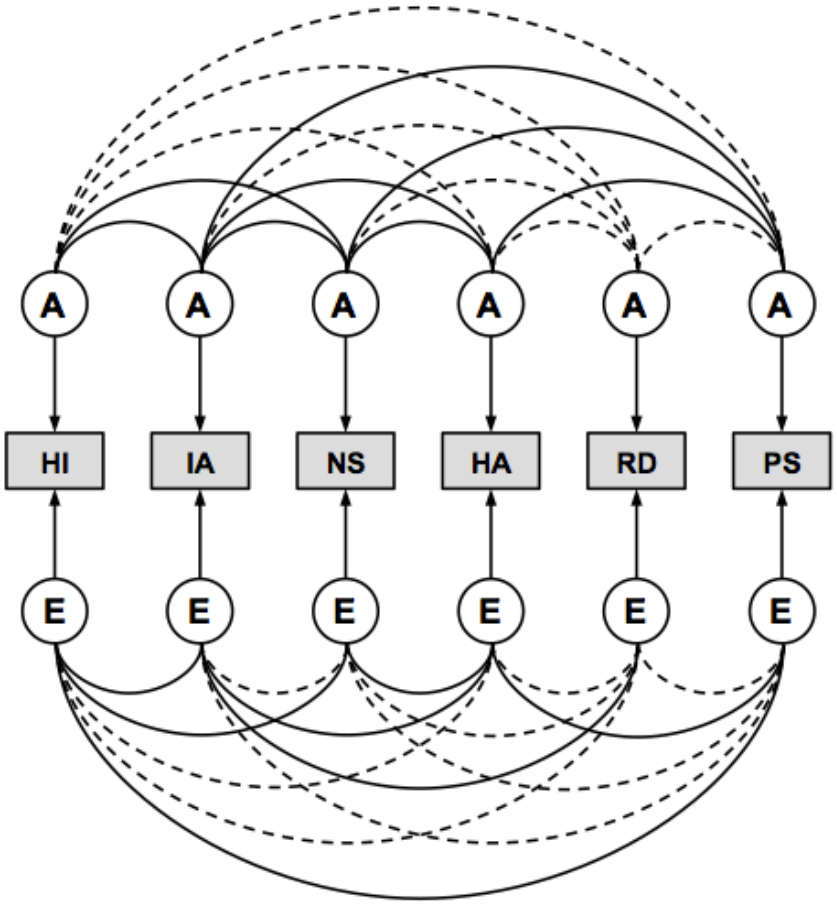
Note: -2LL = log likelihood statistic, df = degrees of freedom, AIC = Akaike's Information Criterion; BIC = Bayesian Information Criterion; χ^2 = likelihood ratio test (LRT) for difference between full and restricted models; Δdf = difference in degrees of freedom for LRT; *p* = significance of LRT; CFS = correlated factors solution.

Table 4.7 Standardised parameter estimates for correlated factors solution of the Cholesky decomposition

	HI	IA	NS	HA	RD	PS
A^2	0.38 (0.29, 0.46)	0.40 (0.31, 0.48)	0.42 (0.33, 0.51)	0.45 (0.36, 0.53)	0.36 (0.26, 0.46)	0.34 (0.24, 0.42)
E^2	0.62 (0.54, 0.71)	0.60 (0.52, 0.69)	0.58 (0.49, 0.67)	0.55 (0.47, 0.64)	0.64 (0.54, 0.74)	0.66 (0.58, 0.76)
Aetiological						
Correlations						
HI	-	0.77 (0.64, 0.90)	0.45 (0.29, 0.60)	0.05 (-0.11, 0.22)	-0.05 (-0.25, 0.15)	0.11 (-0.08, 0.31)
IA	0.32 (0.23, 0.41)	-	0.55 (0.40, 0.72)	0.34 (0.19, 0.49)	0.05 (-0.15, 0.26)	-0.29 (-0.49, -0.10)
NS	0.19 (0.09, 0.28)	0.03 (-0.08, 0.13)	-	-0.28 (-0.43, -0.13)	-0.14 (-0.34, 0.05)	-0.30 (-0.48, -0.12)
HA	-0.01 (-0.11, 0.09)	0.28 (0.18, 0.37)	-0.17 (-0.27, -0.07)	-	0.13 (-0.04, 0.32)	-0.26 (-0.43, -0.08)
RD	-0.07 (-0.17, 0.04)	-0.15 (-0.25, -0.04)	0.06 (-0.05, 0.16)	-0.03 (-0.13, 0.08)	-	0.16 (-0.05, 0.39)
PS	0.12 (0.02, 0.21)	-0.04 (-0.14, 0.06)	-0.09 (-0.19, 0.01)	-0.13 (-0.23, -0.03)	-0.04 (-0.14, 0.06)	-

Note: upper section presents standardised variance components, A^2 = additive genetic; E^2 = non-shared environmental; lower section presents aetiological correlations, with genetic correlations (r_G) above diagonal and non-shared environmental correlations (r_E) below diagonal; HI = hyperactivity-impulsivity, IA = inattention, NS = novelty seeking, HA = harm avoidance, RD = reward dependence, PS = persistence; 95% confidence intervals in parentheses.

Figure 4.1 Path diagram for the best-fitting multivariate model

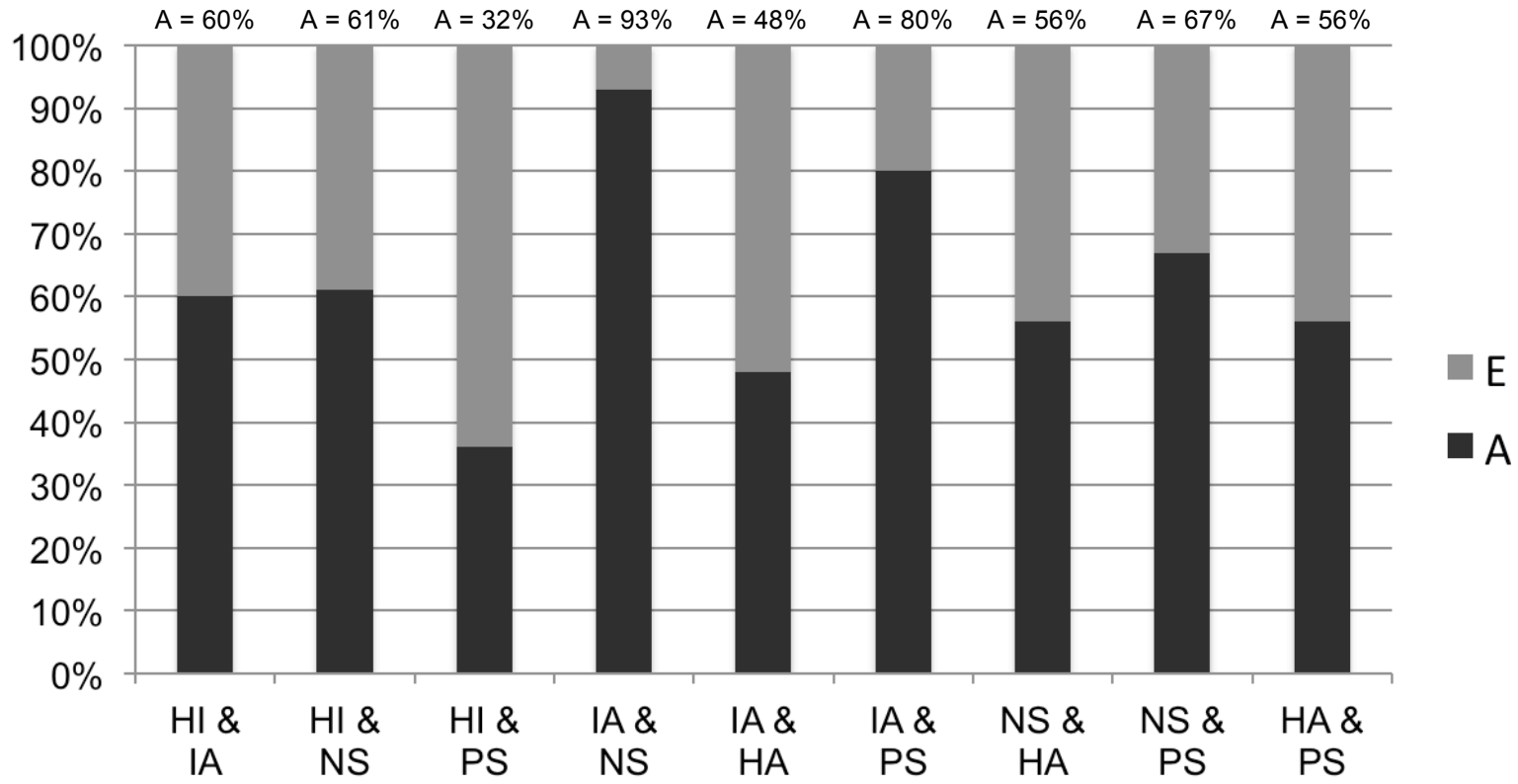


Legend: HI = hyperactivity-impulsivity, IA = inattention, NS = novelty seeking, HA = harm avoidance, RD = reward dependence, PS = persistence; dashed line = non-significant parameter; path diagram depicts genetic and environmental factor loadings and correlations for one twin per pair and thus deviates from the correlated factors solution depicted in chapter 2 (section 2.3.7); parameter estimates presented in Table 4.7.

4.4.5 Bivariate heritabilities

Bivariate heritabilities estimated the proportion of pairwise phenotypic covariances that were attributable to genetic versus non-shared environmental influences. Estimates are presented in Figure 4.2 and were only calculated for variables that were significantly correlated at the phenotypic level (see footnote of Figure 4.2 for equations). For most pairs of variables, genetic influences accounted for around two thirds of phenotypic covariance. Notable exceptions were for hyperactivity-impulsivity with persistence, which was primarily due the non-shared environment; and for inattention with novelty seeking, which was almost entirely due to overlapping genetic influences.

Figure 4.2. Bivariate heritability estimates for variables with significant pairwise associations



Legend: Bivariate heritabilities give the proportion of the pairwise phenotypic covariance between two variables due to additive genetic (A) and non-shared environmental (E) influences; bivariate A = ($\sqrt{A^2}_{VARIABLE\ 1}$ * $\sqrt{A^2}_{VARIABLE\ 2}$ * r_G) /r_{Ph}); bivariate E = ($\sqrt{E^2}_{VARIABLE\ 1}$ * $\sqrt{E^2}_{VARIABLE\ 2}$ * r_E) /r_{Ph}); the proportion due to A is denoted above each column; *denotes non-significant bivariate effect of A based on non-significant genetic correlation in Table 4.7; **denotes non-significant bivariate effect of E based on non-significant non-shared environmental correlation in Table 4.7; HI = hyperactivity-impulsivity; IA = inattention; NS = novelty seeking; HA = harm avoidance; PS = persistence.

Figure 4.3. Path diagrams for the *post-hoc* modelling

Figure 4.3a

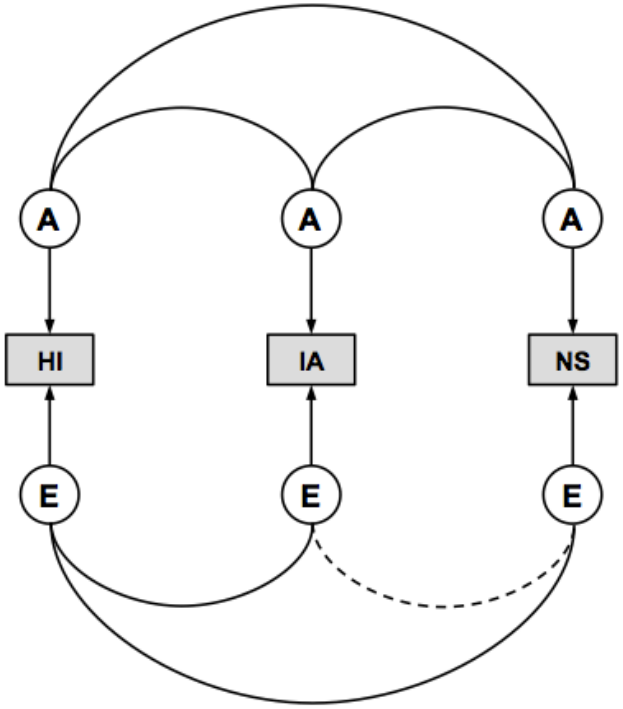
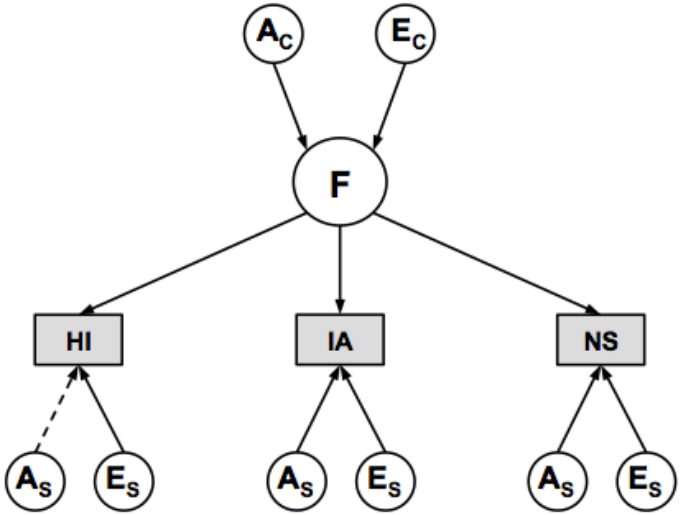


Figure 4.3b



Legend - Figure 4.3a: three variable correlated factors solution; A = additive genetic component; E = non-additive genetic component; HI = hyperactivity-impulsivity; IA = inattention; NS = novelty seeking; dashed line = non-significant loading; path diagram depict factor loadings and correlations for one twin per pair; parameter estimates presented in Table 4.9.

Legend - Figure 4.3b: three variable common pathway model; A_C = additive genetic component for latent factor; E_C = non-shared environmental component for latent factor; F = latent factor; A_S = specific additive genetic component; E_S = specific non-shared environmental component; HI = hyperactivity-impulsivity; IA = inattention; NS = novelty seeking; dashed line = non-significant loading; path diagrams depict factor loadings for one twin pair; estimates presented in Table 4.9.

4.4.6 *Post-hoc* analyses of novelty seeking

ADHD symptoms of hyperactivity-impulsivity and inattention were both significantly genetically correlated with the temperament dimension of novelty seeking. One explanation is that there are unique genetic associations of novelty seeking with hyperactivity-impulsivity versus inattention; alternatively a single genetic factor could account for covariance between all three phenotypes; finally, the covariance between the three phenotypes may be best represented by a latent factor that has its own genetic influence. To test these hypotheses the fit of a three variable (trivariate) correlated factors solution was compared to that of trivariate independent and common pathway models. In the independent pathway model a single set of genetic and environmental factors account for phenotypic covariance; in the common pathway model a latent factor accounts for phenotypic covariance and is influenced by a single set of genetic and environmental factors (section 2.3.7). Fit statistics for all models are presented in Table 4.8. Based on the AIC statistic, the best fitting model is the Cholesky decomposition, however using the BIC statistic the common pathway model provides a better fit. Both models are therefore interpreted.

The correlated factors solution of the Cholesky decomposition was interpreted first. Fit statistics (Table 4.8) indicated that the AE model provided a worse fit to the data than the full ADE model (χ^2 = 14.90, p = 0.02); however this difference is non-significant if adopting an adjusted threshold of $p < .01$ to account for the multiple models fit in these analyses (as in other research, e.g. Wood et al., 2011a). Interpretation of the AE model also ensures parity with the multivariate model described in section 4.4.4. The model is depicted in Figure 4.3a and parameter estimates are presented in Table 4.9. Estimates are in line with those derived from the six-variable model (section 4.4.4), indicating that both dimensions of ADHD were similarly associated with novelty seeking.

The common pathway model (Figure 4.3b, Table 4.10) was then interpreted. The fit of an AE model was not significantly different to that of the full ADE model (χ^2 = 7.55, p = 0.11). In the AE model a common latent factor accounted for 58% of the variance in hyperactivity-impulsivity, 42% of the variance in inattention and 14% of the variance in novelty seeking. This latent factor was

moderately heritable (61%), indicating that around two-thirds of the phenotypic covariance was accounted for by shared genetic effects. The remaining variance unique to each phenotype was accounted for by residual additive genetic (A_S) and non-shared environmental (E_S) influences. However the loading of the common factor onto novelty seeking was significantly lower than the loading onto hyperactivity-impulsivity or inattention, as denoted by the non-overlapping confidence intervals for estimates of F^2.

Table 4.8 Fit statistics for the *post-hoc* modelling

Model	Parameters	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated	-	14335.48	4809	4717.48	-9361.27	-	-	-
CFS	ADE	14395.64	4842	4711.64	-9444.61	-	-	-
	AE	14410.55	4848	4714.55	-9457.78	14.91	6	<.05
IP	ADE	14398.11	4842	4714.11	-9443.38	-	-	-
	AE	14411.02	4848	4715.02	-9457.55	12.91	6	<.05
CP	ADE	14410.79	4846	4718.79	-9450.79	-	-	-
	AE	14418.33	4850	4718.33	-9460.77	7.55	4	0.11

Note: -2LL = log likelihood statistic, df = degrees of freedom, AIC = Akaike's Information Criterion; BIC = Bayesian Information Criterion; χ^2 = likelihood ratio test (LRT) for difference between full and restricted models; Δdf = difference in degrees of freedom for LRT; *p* = significance of LRT; CFS = correlated factors solution; IP = independent pathway; CP = common pathway.

Table 4.9 Standardised parameter estimates for the *post-hoc* Cholesky decomposition

	HI	IA	NS
A^2	0.38 (0.29, 0.46)	0.41 (0.32, 0.49)	0.41 (0.32, 0.50)
E^2	0.62 (0.54, 0.71)	0.59 (0.51, 0.68)	0.59 (0.50, 0.68)
Correlations			
HI	-	0.75 (0.62, 0.88)	0.44 (0.28, 0.60)
IA	0.33 (0.24, 0.41)	-	0.56 (0.40, 0.72)
NS	0.19 (0.09, 0.29)	0.03 (-0.08, 0.13)	-

Note: upper section presents variance components, A^2 = additive genetic; E^2 = non-shared environmental; lower section presents correlations, with genetic correlations (rG) above diagonal and non-shared environmental correlations (rE) below diagonal; HI = hyperactivity-impulsivity; IA = inattention; NS = novelty seeking; 95% confidence intervals in parentheses.

Table 4.10 Standardised parameter estimates for the *post-hoc* common pathway model

	F	HI	IA	NS
A_C^2	0.61 (0.48, 0.73)	-	-	-
E_C^2	0.39 (0.27, 0.52)	-	-	-
F^2	-	0.58 (0.48, 0.71)	0.42 (0.34, 0.50)	0.14 (0.10, 0.18)
A_S^2	-	0.04 (0.00, 0.13)	0.13 (0.04, 0.22)	0.30 (0.21, 0.38)
E_S^2	-	0.38 (0.28, 0.47)	0.45 (0.37, 0.54)	0.56 (0.48, 0.65)

Note: F = latent factor; A_C^2 = additive genetic component for latent factor; E_C^2 = non-shared environmental component for latent factor; A_S^2 = specific additive genetic component; E_S^2= specific non-shared environmental component; F^2 = latent factor loading; HI = hyperactivity-impulsivity; IA = inattention; NS = novelty seeking; 95% confidence intervals in parentheses.

4.5 DISCUSSION

This study examined the association of ADHD symptoms of hyperactivity-impulsivity and inattention with Cloninger's temperament dimensions in adults. Both ADHD symptom domains were significantly associated with novelty seeking. *Post-hoc* analyses revealed that the covariance among these dimensions could be represented via a single latent factor that was around 60% heritable. There were also differential associations of the two ADHD symptom dimensions with harm avoidance and persistence. Harm avoidance was uniquely correlated with inattention at the phenotypic, genetic and environmental levels. Persistence was phenotypically correlated with both ADHD dimensions but with opposite directions of association: a positive association with hyperactivity-impulsivity was driven primarily by overlapping non-shared environmental influences; while a negative association with inattention was primarily due to overlapping genetic influences. However because phenotypic correlations were weak, persistence may be of only limited relevance when characterising ADHD.

The results reported confirm previously observed phenotypic associations of total ADHD symptoms with increased novelty seeking, of inattentive symptoms with increased harm avoidance, and of hyperactive-impulsive symptoms with increased persistence (Gomez et al., 2012, Salgado et al., 2009). They also extend previous studies by providing estimates of the degree to which genetic and environmental factors drive these associations. Bivariate heritabilities indicated that for most pairwise associations, genetic factors were more

important than the non-shared environment. Although previous twin studies have identified genetic associations between ADHD and novelty seeking in children (Wood et al., 2011a, Young et al., 2009b, Young et al., 2000), this is the first study to examine all of Cloninger's temperament dimensions in relation to ADHD, the first to focus on ADHD in adults, and the first to fully explore the differential associations of temperament with the two ADHD domains. There are a number of theoretical and clinical implications that should be considered.

The first consideration is that the differential association of Cloninger's temperament dimensions with the ADHD symptom domains is consistent with a bi-factor model of ADHD. This model is based on increasing evidence supporting the separation of aetiological processes into those that influence a general ADHD factor, consisting of hyperactivity-impulsivity and inattention, and those that influence each of the two clinical domains separately (Toplak et al., 2009, Toplak et al., 2012). The bi-factor approach has already been applied phenotypically to examine childhood data on ADHD and the 'Big Five' personality dimensions (Martel et al., 2011). Results indicated that specific inattention was associated with introversion and agreeableness, whereas specific hyperactivity-impulsivity was associated with extraversion.

The phenotypic and genetic modelling reported in the present study extends this approach into adulthood, suggesting that novelty seeking is related to a general ADHD factor, while harm avoidance is uniquely related to an inattentive factor. Because of conceptual overlaps between Cloninger's temperament dimensions and the Five Factor model of personality (Bouchard Jr and Loehlin, 2001), the present findings can be seen as supporting those of previous research. However, it should be noted that the *post-hoc* genetic modelling identified a relatively weak association of novelty seeking with a common latent factor comprising the core symptoms of ADHD. This suggests that while novelty seeking may be related to the core symptoms of ADHD, it is also influenced by unique aetiological factors.

The implications of this first point are that genetically homogeneous ADHD subtypes could be established by examining individual differences in temperament. This has a knock-on effect for molecular genetic and

neurobiological studies in providing a potentially useful approach to addressing some of the issues of heterogeneity that have dogged research into ADHD. For example, high inattention and harm avoidance might characterise a genetically homogenous ADHD subgroup, different from a subgroup with high symptoms of hyperactivity-impulsivity, inattention and novelty seeking. Evidence to support this hypothesis comes from the fact that there were only weak genetic correlations between novelty seeking and harm avoidance in the present study. Mapping common genetic variants to ADHD subgroups defined on the basis of temperament profiles might therefore prove more fruitful than attempting to map genes to a phenotypically heterogeneous group of adults with ADHD.

Similarly, it might also prove beneficial to map genes to specific temperament traits within ADHD. This is consistent with the results of a recent candidate gene study (de Cerqueira et al., 2011), in which different markers were linked to distinct profiles of temperament in a large clinical sample of adults with ADHD. A related implication is that temperament dimensions might be viewed as putative endophenotypes for ADHD (Nigg et al., 2004b, Nyman et al., 2012, Reif et al., 2011b); however, a key criterion for endophenotypes is that they provide a simplified measure of other, more complex traits (Gottesman and Gould, 2003). Because temperament is assessed using behavioural rating scales it is unclear whether it represents a simpler phenotype than the existing behavioural measures of ADHD. The modest genetic correlations between the different temperament dimensions do suggest a low level of genetic complexity, although the moderate heritability estimates indicate that temperament is not more strongly influenced by genes than are the symptoms of ADHD.

The second consideration is that unique profiles of temperament might also characterise the heterogeneous expression of psychiatric comorbidity in ADHD. This is consistent with a person-centered approach to heterogeneity, whereby different personality profiles have been found to distinguish between homogeneous ADHD subgroups that differ with regard to comorbidity (Martel et al., 2010a). Theoretically, the results of the present study could also be informative with regard to comorbidity patterns. For example, novelty seeking refers to behaviours such as exploratory excitability, impulsive decision-making and quick loss of temper, which may index other externalising traits in addition

to ADHD. Indeed, Young et al. (2000, 2009) found that a heritable latent phenotype accounted for covariance between ADHD, novelty seeking, conduct problems and substance use. The results from the present study and previous research therefore converge to suggest that individuals high in novelty seeking may be more likely to experience ADHD and comorbid symptoms of behavioural disinhibition due to a common genetic liability. These findings could now be extended to examine emotional lability, which refers to chronic symptoms of irritability and mood volatility that are correlated with the core dimensions of ADHD (Barkley et al, 2010; Skirrow et al, 2009). It therefore seems plausible that certain temperamental profiles such as high novelty seeking might also identify emotionally reactive individuals with ADHD. This is a future direction for research.

Conversely, the dimension of harm avoidance refers to pessimistic worry and avoidance behaviours. Results from this study therefore suggest that there may be an increased genetic risk for internalising symptoms among adults who are high in inattentive symptoms only. This is consistent with clinical studies linking the inattentive subtype of ADHD with internalising problems such as anxiety disorders (Acosta et al., 2008). These arguments are theoretical and future research should therefore examine the extent to which unique temperament profiles moderate or mediate genetic and environmental associations between ADHD and psychiatric comorbidity.

The third consideration concerns developmental pathways between temperament and ADHD. It has previously been argued that temperament traits manifest prior to ADHD during development (Taurines et al., 2010), meaning that different traits might characterise causal pathways leading to ADHD (Nigg et al., 2004b). The present study suggests that there is a genetic basis for such a hypothesis; however the direction of causation remains unclear, with one childhood study suggesting that there are causal paths from ADHD to novelty seeking and not vice-versa (Wood et al., 2011a). Further longitudinal studies are therefore required to determine the developmental relationship between temperament and ADHD, while accounting for innovation and stability in genetic and environmental effects.

Finally, it is possible to comment on the heritability of ADHD symptoms and temperament dimensions in relation to existing literature. The results confirm previous twin studies examining self-ratings of adult ADHD, estimating moderate heritability for hyperactivity-impulsivity (37%) and inattention (40%). The present study used self-ratings of ADHD because temperament was also self-reported; however, higher heritability for adult ADHD symptoms has previously been found in the same population that we report on here when using a composite of self and parent ratings (Chang et al., 2013). Despite the use of self-ratings, the phenotypic and genetic correlations between hyperactivity-impulsivity and inattention are similar to those reported previously (Greven et al., 2011c, Larsson et al., 2012b, McLoughlin et al., 2007).

The present findings also confirm previous twin studies of Cloninger's temperament dimensions; estimating moderate heritability for novelty seeking (42%), harm avoidance (45%), reward dependence (36%) and persistence (34%) (Ando et al., 2002, Ando et al., 2004, Gillespie et al., 2003, Heath et al., 1994, Keller et al., 2005, Stallings et al., 1996). The low-to-modest phenotypic and genetic correlations among these dimensions suggest that they are largely independent, with only a small degree of overlap in their aetiologies.

The findings should be interpreted in the context of several limitations. First, there were low phenotypic correlations between ADHD symptoms and some temperament dimensions, in particular reward dependence. The inclusion of reward dependence in genetic models was nonetheless important in demonstrating that it was aetiologically unrelated to ADHD. Second, ADHD and temperament were examined cross-sectionally in adult twins, meaning that it was not possible to evaluate developmental-genetic associations or to account for the stability of dimensions during development (i.e. from childhood onwards). Accordingly, the ADHD symptoms measured here might reflect manifestations of alternative phenotypes rather than chronic symptoms of ADHD.

Third, temperament was measured in accordance with Cloninger's psychobiological model of personality, yet the dimensions of character were not studied. Character refers to later-emerging aspects of personality that have

also been linked to ADHD, although the results from phenotypic studies have been less consistent than those reported for the dimensions of temperament (Anckarsäter et al., 2006, Cho et al., 2008a, Lynn et al., 2005, Smalley et al., 2009, Tillman et al., 2003). Fourth, there were two potential issues regarding the measurement of ADHD and temperament in this study. One was that the use of self-ratings for all measures might have led to shared rater variance, which could have inflated the correlations between measures. Another potential problem is that of item overlap between Cloninger's temperament dimensions and the symptoms of ADHD; however examination of the questionnaires indicated that identical items did not appear across measures.

5. AETIOLOGICAL ASSOCIATIONS BETWEEN THE SYMPTOMS OF ADHD AND EMOTIONAL LABILITY

5.1 OVERVIEW

ADHD and emotional lability frequently co-occur in clinical settings. The aim of chapter 5 was to examine their association in a general population sample and to decompose phenotypic covariation into genetic and environmental components. Participants were 1,920 child and adolescent twin pairs aged 5-18 years. ADHD symptoms of hyperactivity-impulsivity and inattention were assessed using a modified version of the DuPaul rating scale, completed by parents. Symptoms of emotional lability were assessed using the parent-rated Conners 10-item scale. Multivariate structural equation modelling revealed that a common pathway model best accounted for the covariance between dimensions, represented by a highly heritable latent factor. *Ad-hoc* analyses identified unique genetic associations of emotional lability with inattention (after controlling for hyperactivity-impulsivity) and with hyperactivity-impulsivity (after controlling for inattention); and revealed a significantly stronger association of emotional lability with the common latent factor in older individuals. This supports prior contentions that emotional lability is strongly related to ADHD.

5.2 INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is characterised by age-inappropriate and impairing symptoms across two core dimensions: hyperactivity-impulsivity and inattention. Diagnostic criteria additionally recognise symptoms of emotional lability as an associated feature of ADHD (American Psychiatric Association, 2000), although the extent of the phenotypic and aetiological associations with symptoms of hyperactivity-impulsivity and inattention remains unclear. Understanding these associations is important in determining the nature of the relationship that emotional lability has with ADHD.

Emotional lability refers to a set of symptoms including irritability, low frustration tolerance, temper outbursts and mood volatility. One hypothesis is that emotional lability is an integral feature of ADHD (Barkley, 2010, Skirrow et al., 2009), based on evidence of strong phenotypic associations between emotional lability and ADHD symptoms in clinical samples of children and adults, in addition to links between emotional lability and functional impairments (Anastopoulos et al., 2011, Barkley and Fischer, 2010, Skirrow and Asherson, 2013). These findings are consistent with historical definitions of the disorder, which included emotional lability as a core clinical feature (Barkley, 2010).

One consistent line of evidence to suggest a common aetiology comes from treatment studies documenting a concomitant decline in symptoms of hyperactivity-impulsivity, inattention and emotional lability in response to methylphenidate and atomoxetine in adults (Marchant et al., 2011a, Marchant et al., 2011b, Reimherr et al., 2005b, Reimherr et al., 2007, Rosler et al., 2010). Similar results have been reported in child and adolescent samples, where there is evidence of a concomitant decline in ADHD symptoms and aggression-related behaviours in response to stimulant and non-stimulant medication (Connor et al., 2002, Nevels et al., 2010). The co-action of drug treatments on symptoms of emotional lability and ADHD, and in particular their strong co-variation during the treatment response, might therefore reflect a common aetiology operating at the neurobiological level.

Family studies provide another line of evidence and suggest that familial factors may account for the covariation of emotional lability symptoms and ADHD. Some studies indicate a tendency for symptoms to co-segregate among the first-degree relatives of children and adults with ADHD, interpreted as evidence of a distinct familial subtype referred to as deficient emotional self-regulation (Biederman et al., 2012d, Surman et al., 2011). However, familial co-segregation is also consistent with shared aetiological influences acting on the symptoms of emotional lability and ADHD at the population level. In contrast another family study, using a large sample of children and adolescents, found that although there was a familial risk for emotional lability, there was no significant co-segregation with ADHD symptoms in unaffected siblings (Sobanski et al., 2010). This study further found that the phenotypic association

between emotional lability and ADHD was primarily with hyperactive-impulsive symptoms.

The results across treatment and family studies therefore suggest that the symptoms of emotional lability and ADHD may arise as a result of a common aetiology. However, these results are not conclusive and are subject to limitations. First, because participants for these studies were typically ascertained from specialist clinics, the samples may be subject to referral bias. This also means that the association between symptoms of emotional lability and ADHD within the general population is poorly understood. Second, the family studies that identified significant co-segregation of emotional lability and ADHD symptoms were unable to partition the familial risk into genetic versus shared-environmental components, leaving open the question of whether familial co-segregation is driven by genetic or environmental factors.

Community twin designs, unselected for phenotypic extremes, provide a robust, alternative strategy for evaluating the aetiological association between symptoms of emotional lability and ADHD. Twin studies consistently estimate high heritability (70-80%) for symptoms of hyperactivity-impulsivity and inattention (Nikolas and Burt, 2010) and have revealed a substantial overlap in genetic influences between the two dimensions (Greven et al., 2011a, Larsson et al., 2013, McLoughlin et al., 2007). The heritability of constructs pertaining to emotional lability is moderate-to-high (50-70%) (Boomsma et al., 2006, Hudziak et al., 2005, van Beijsterveldt et al., 2004, Volk and Todd, 2007); however no twin studies to date have directly examined the aetiological association between symptoms of emotional lability and ADHD.

The aim of the present study was to address these gaps in the literature using a multivariate twin modelling design. Phenotypic correlations were initially examined to test the hypothesis that emotional lability is more strongly related to symptoms of hyperactivity-impulsivity than inattention (Sobanski et al., 2010) and to determine the degree of phenotypic overlap within a large, unselected sample of children and adolescents. The extent to which common aetiological influences accounted for phenotypic associations was then explored. It was hypothesised that there would be an aetiological overlap between symptoms of

emotional lability and ADHD. However, by comparing the fit of different multivariate twin models it was possible to assess whether genetic and environmental influences across dimensions were correlated or overlapping (indicating a common aetiology but not necessarily supporting the hypothesis that emotional lability is a core component of ADHD), versus whether common genetic and environmental influences across dimensions were accounted for via a single higher-order latent factor (indicating that emotional lability is an integral feature of a broader phenotype that comprises the core symptoms of ADHD, with a common aetiology). Finally, *ad-hoc* analyses were conducted to test for unique aetiological associations of emotional lability with the two dimensions of ADHD and to test for possible effects of age.

5.3 METHOD

5.3.1 Sample and measures

The sample was from the Cardiff Study of all Wales and North West of England Twins (CASTANET). A total of 3,840 individuals from 1,920 twin pairs were included in analyses: 348 monozygotic males (MZM), 383 monozygotic females (MZF), 276 dizygotic males (DZM), 313 dizygotic females (DZF), and 600 dizygotic opposite-sex (DZO) pairs. Participating twins were aged 5-18 years (mean = 11.20 years, SD = 3.09). ADHD symptoms of hyperactivity-impulsivity and inattention were rated by the mothers of twins using a modified version of the DuPaul Rating Scale, adapted to include the full 18-items outlined in DSM-IV (DuPaul, 1981, Thapar et al., 2000). Emotional Lability was assessed using the parent-rated Conner's 10-item scale, also completed by the mothers of twins (Conners et al., 1998a). The separation of hyperactivity-impulsivity, inattention and emotional lability is supported via factor-analytic research (Parker et al., 1996, Westerlund et al., 2009), including in this sample (Chen, unpublished data). The sample and measures are described in detail in section 2.2.3.

5.3.2 Statistical analyses

Preliminary analyses were conducted in Stata version 10.1 (StataCorp., 2007). Structural equation modelling was conducted using Mx (Neale et al., 2006). Prior to modelling, raw scores for each dimension were square-root transformed to normalise the data distributions (in Stata: skewness=0±1, kurtosis=3±1) and regressed to control for age and sex effects (see section 2.3.4).

Cross-twin within-trait, cross-twin cross-trait and phenotypic correlations provided estimates of phenotypic covariation and an overview of the data for genetic analyses. All correlations were estimated using a constrained saturated model fit in Mx (section 2.3.5). Univariate sex-limited models decomposed phenotypic variances in into genetic and environmental components while testing for aetiological sex differences. These models parameterised additive genetic (A) and non-shared environmental (E) components of variance, in addition to either shared-environmental (C) or non-additive genetic (D) components depending on the pattern of twin correlations observed. Models including a contrast effect (*b*) parameter were also fit when low cross-twin within-trait correlations were observed for DZ twin pairs in the presence of greater variances for DZ than MZ twins, since this is indicative of possible rater contrast effects. ADE and ADE-*b* models were tested separately, since this provides greater power to detect genetic non-additivity (Rietveld et al., 2003).

To determine the extent to which phenotypic covariance was due to genetic and environmental influences, the fit of three multivariate models was compared (see section 2.3.7): The triangular (Cholesky) decomposition, from which the mathematically equivalent correlated factors solution was interpreted (Figure 2.5, section 2.3.7); the independent pathway model (Figure 2.6, section 2.3.7); and the common pathway model (Figure 2.7, section 2.3.7).

Ad-hoc structural equation modelling was used to address two additional questions. First, *ad-hoc* modelling tested for unique aetiological associations of emotional lability with the two dimensions of ADHD. Inattention was regressed to control for hyperactivity-impulsivity and tested for aetiological associations with emotional lability using a bivariate Cholesky decomposition, from which the

correlated factors solution was interpreted. The same method was then applied to test for unique aetiological associations between hyperactivity-impulsivity and emotional lability after controlling for inattention. Second, *ad-hoc* modelling tested whether the relationships between hyperactivity-impulsivity, inattention and emotional lability differed as a function of age. The full sample was split in two around the mean age of all participating twins, resulting in two age cohorts (age range 5-10 years, mean age = 8.30, SD = 1.30, n = 880 pairs; age range 11-18 years, mean age = 13.56, SD = 1.88, n = 1,040 pairs). The main multivariate modelling was then repeated in each cohort.

5.4 RESULTS

5.4.1 Descriptive statistics

Descriptive statistics are presented in Table 5.1. Tests of mean differences were performed on the raw data, using robust regressions in Stata to control for dependence in the observations from twin pairs (Williams, 2000). Mean scores were significantly higher for males than females for the symptoms of hyperactivity-impulsivity (t = 10.00, p < .001), inattention (t = 10.90, p < .001) and emotional lability (t = 2.36, p < .05). Younger age was also significantly associated with higher mean scores for these phenotypes (respectively: t = -10.40, p < .001; t = -2.21, p < .05; t = -4.63, p < .001).

Table 5.1 Descriptive statistics for all variables

	Mean (Standard Deviation)					
	All	MZM	MZF	DZM	DZF	DZO
HI	5.73 (5.83)	6.53 (5.80)	4.64 (4.77)	6.98 (6.67)	4.87 (5.48)	5.84 (6.04)
IA	6.04 (6.26)	6.68 (6.12)	4.60 (5.14)	7.63 (7.04)	5.23 (6.02)	6.28 (6.48)
EL	2.33 (2.81)	2.16 (2.68)	2.08 (2.57)	2.57 (3.00)	2.29 (2.78)	2.49 (2.92)

Note: descriptive statistics reported for raw data; HI = hyperactivity-impulsivity; IA = inattention; EL = emotional lability; All = statistics reported for whole sample; MZM = monozygotic males; MZF = monozygotic females; DZM = dizygotic same-sex males; DZF = dizygotic same-sex females; DZO = dizygotic opposite-sex twins.

Equality of variances across sex and zygosity groups was assessed using Levene's test. Phenotypic variances were significantly greater for males than females for hyperactivity-impulsivity (F = 132.48, p < .001), inattention (F = 20.71, p < .001) and emotional lability (F = 85.12, p < .001), suggesting scalar sex differences. Variances were also significantly greater for DZ than MZ twins for all phenotypes (respectively: F = 20.32, p < .001; F = 27.74, p < .001; F = 26.76, p < .001). This latter finding could indicate contrast effects. All variance differences were confirmed using the saturated model, testing whether variances for each trait could be constrained across sex or zygosity. In all instances these constraints led to a significant deterioration in model fit based on the likelihood ratio test (p < 0.01, respectively).

5.4.2 Correlations

Phenotypic correlations (95% confidence intervals) were 0.70 (0.62, 0.72) between hyperactivity-impulsivity and inattention, 0.63 (0.61, 0.65) between hyperactivity-impulsivity and emotional lability, and 0.58 (0.56, 0.60) between inattention and emotional lability. Two interesting findings emerge. First, non-overlapping confidence intervals indicate a significantly stronger association of emotional lability with hyperactivity-impulsivity than with inattention. Second, overlapping confidence intervals indicate that hyperactivity-impulsivity is as strongly related to emotional lability as it is to inattention.

Twin correlations are presented by sex and zygosity in Tables 5.2 and 5.3. For hyperactivity-impulsivity and novelty seeking, DZ cross-twin within-trait correlations (Table 5.2) were less than half the MZ correlations. This suggests D influences on phenotypic variance, or in tandem with the significantly lower variances for MZ than DZ pairs reported above could indicate rater contrast effects (*b*). For emotional lability, the pattern of correlations for males was similar, suggesting effects of D and/or *b*; however for females the DZ cross-twin within-trait correlations were more than half of those estimated for MZ pairs, suggesting possible influences of C. Cross-twin cross-trait correlations (Table 5.3) were generally higher for MZ pairs than DZ pairs, suggesting mainly A influences on phenotypic covariance but with possible C influences on the covariance between ADHD symptoms and emotional lability in females.

Table 5.2 Cross-twin within-trait correlations

	MZM	MZF	DZM	DZF	DZO
HI	0.77 (0.74, 0.81)	0.74 (0.70, 0.78)	0.22 (0.11, 0.33)	0.29 (0.19, 0.39)	0.20 (0.13, 0.27)
IA	0.66 (0.60, 0.71)	0.66 (0.60, 0.71)	0.13 (0.02, 0.23)	0.29 (0.19, 0.38)	0.18 (0.11, 0.25)
EL	0.62 (0.56, 0.68)	0.71 (0.66, 0.76)	0.24 (0.13, 0.34)	0.42 (0.33, 0.50)	0.26 (0.19, 0.33)

Note: cross-twin within-trait correlations presented by sex and zygosity; correlations performed on transformed data regressed on age and sex; HI = hyperactivity-impulsivity; IA = inattention; EL = emotional lability; MZM = monozygotic males; MZF = monozygotic females; DZM = dizygotic same-sex males; DZF = dizygotic same-sex females; DZO = dizygotic opposite-sex twins; 95% confidence intervals in parentheses.

Table 5.3 Cross-twin cross-trait correlations

	MZM	MZF	DZM	DZF	DZO
HI & IA	0.57 (0.53, 0.61)	0.60 (0.56, 0.63)	0.19 (0.10, 0.28)	0.29 (0.21, 0.37)	0.24 (0.18, 0.30)
HI & EL	0.53 (0.49, 0.56)	0.55 (0.52, 0.59)	0.23 (0.14, 0.31)	0.36 (0.28, 0.43)	0.21 (0.15, 0.27)
IA & EL	0.44 (0.40, 0.49)	0.50 (0.46, 0.54)	0.23 (0.15, 0.32)	0.37 (0.29, 0.43)	0.24 (0.18, 0.29)

Note: cross-twin cross-trait correlations presented by sex and zygosity; correlations performed on transformed data regressed on age and sex; HI & IA = correlation of hyperactivity-impulsivity for twin 1 with inattention for twin 2; HI & EL = correlation of hyperactivity-impulsivity for twin 1 with emotional lability for twin 2; IA & EL = correlation of inattention for twin 1 with emotional lability for twin 2; MZM = monozygotic males; MZF = monozygotic females; DZM = dizygotic same-sex males; DZF = dizygotic same-sex females; DZO = dizygotic opposite-sex twins; 95% confidence intervals in parentheses.

5.4.3 Univariate sex-limited modelling

Full sex-limitation models confirmed the presence of significant variance (scalar) sex differences between males and females for the symptoms of hyperactivity-impulsivity and inattention. For both phenotypes, the best-fitting models parameterised AE influences and additionally included a contrast effect (*b*) that could be equated for males and females. For emotional lability, a hybrid model was fit on the basis of the observed within-twin correlations. The full hybrid model enabled C influences on emotional lability in females but D and/or *b* influences in males. This model is plausible since C and D were never estimated simultaneously for the same twin pair. The respective influences of C and D were non-significant and a scalar sex differences model that parameterised AE, in addition to *b* for boys only, provided the best fit to the data. Fit statistics are presented in Appendix C. Parameter estimates for the best-fitting models are presented in Table 5.4. Heritability estimates were 83% for hyperactivity-impulsivity, 77% for inattention and 71% for emotional lability.

Table 5.4 Standardised parameter estimates for the best-fitting univariate models

	A^2	E^2	*b*
HI	0.83 (0.81, 0.86)	0.17 (0.14, 0.19)	-0.11 (-0.14, -0.08)
IA	0.77 (0.73, 0.81)	0.23 (0.19, 0.27)	-0.11 (-0.15, -0.08)
EL	0.71 (0.67, 0.75)	0.29 (0.25, 0.33)	-0.07 (-0.12, -0.02)*

Note: A^2 = standardised additive genetic variance component; E^2 = standardised non-shared environmental variance component; *b* = contrast effect; HI = hyperactivity-impulsivity; IA = inattention; EL = emotional lability; * *b* for emotional lability included for boys only; 95% confidence intervals in parentheses.

5.4.4 Multivariate modelling

Based on the univariate results, all multivariate models were specified with a scalar to account for the greater phenotypic variances in males' scores. These models parameterised ADE in addition to contrast effects (*b*), with *b* included for males only for *EL*. There was no evidence from the univariate modelling to support inclusion of C in the multivariate models. The AIC fit statistic (Table 5.5) did not indicate a preference for any single class of model; however the BIC statistic indicated a strong preference for the common pathway model (see

section 2.3.4). The common pathway model parameterised common influences of ADE on the latent factor (A_C, D_C, E_C) in addition to influences that were specific to each dimension (A_S, D_S, E_S). Of these, only D_C could be dropped without a significant deterioration in fit (Table 5.5). Parameter estimates for the best-fitting model are presented in Table 5.6 and the path diagram in Figure 5.1. Note that although estimates of A_S were non-significant, they were retained in the best-fitting model as it is considered biologically implausible to find D without A (Plomin et al., 2008).

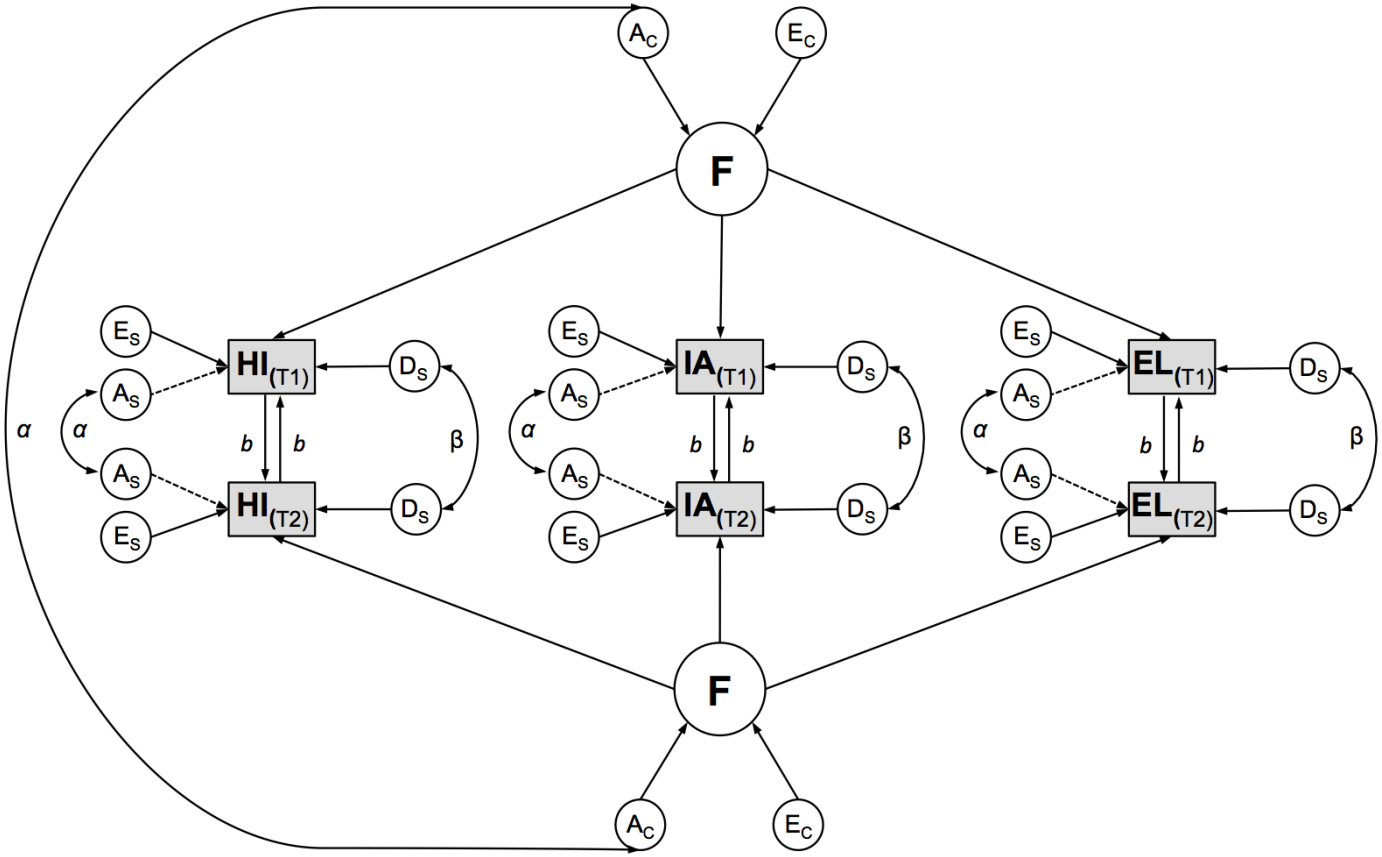
In the best-fitting model, phenotypic covariation was represented by a highly heritable common latent factor (A_C^2=0.89) that accounted for 77% of the total variance in hyperactivity-impulsivity, 67% in inattention and 53% in emotional lability. Genetic influences operating on the common latent factor thus accounted for 69% of the total variance in hyperactivity-impulsivity, 60% in inattention and 47% in emotional lability (see Table 5.7 for percentages and calculations). There were also specific genetic influences on each phenotype, which were from non-additive genetic sources. These accounted for an additional 14% of the variance in hyperactivity-impulsivity, 18% in inattention and 25% in emotional lability. The remaining variance in hyperactivity-impulsivity, inattention and emotional lability was explained by non-shared environmental influences, primarily operating at the specific level (E_S). These findings demonstrate that covariation between the three dimensions was primarily due to shared genetic effects.

Table 5.5 Fit statistics for the multivariate models

Model	Parameters	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated	-	24684.16	11463	1758.16	-30988.52	-	-	-
CFS	A, D, E, r_A, r_D, r_E, *b*	24751.01	11490	1771.01	-31057.17	-	-	-
IP	A_C, D_C, E_C, A_S, D_S, E_S, *b*	24751.11	11490	1771.11	-31057.11	-	-	-
CP	A_C, D_C, E_C, A_S, D_S, E_S, *b*	24759.85	11494	1771.85	-31067.86	-	-	-
CP	**A_C, E_C, A_S, D_S, E_S, *b***	**24759.85**	**11495**	**1769.85**	**-31071.64**	**0.00**	**1**	**1.00**
CP	A_C, D_C, E_C, A_S, E_S, *b*	24814.48	11497	1820.48	-31051.88	54.63	3	<.001
CP	A_C, E_C, A_S, E_S, *b*	24814.48	11498	1818.48	-31055.66	54.63	4	<.001
CP	A_C, D_C, E_C, A_S, D_S, E_S, *b**	24820.03	11495	1830.03	-31041.55	60.18	1	<.001
CP	A_C, D_C, E_C, A_S, D_S, E_S, *b***	24820.04	11495	1830.04	-31041.55	60.18	1	<.001
CP	A_C, D_C, E_C, A_S, D_S, E_S, *b****	24768.14	11495	1778.14	-31067.49	8.29	1	<.001
CP	A_C, D_C, E_C, A_S, D_S, E_S	24849.81	11497	1855.81	-31034.22	89.96	3	<.001
CP	A_C, E_C, A_S, D_S, E_S	24861.17	11498	1865.17	-31032.32	101.32	4	<.001
CP	A_C, D_C, E_C, A_S, E_S	24927.93	11500	1927.93	-31006.50	168.08	6	<.001
CP	A_C, E_C, A_S, E_S	24944.01	11501	1942.01	-31002.24	184.16	7	<.001

Note: -2LL = log likelihood statistic; df = degrees of freedom; AIC = Akaike's Information Criterion; BIC = Bayesian Information Criterion; χ^2 = likelihood ratio test (LRT) for difference between full and restricted models; Δdf = difference in degrees of freedom for LRT; *p* = significance of LRT; CFS = correlated factors solution of the Cholesky decomposition; IP = independent pathway model; CP = common pathway model; all models constrained male variances to be a scalar multiple of female variances for parent, teacher and self ratings; for EL contrast effects (*b*) were included for males only; *dropped the contrast effect for HI; **dropped the contrast effect for IA; ***dropped the contrast effect for EL; best-fitting model denoted in **bold**.

Figure 5.1. Path diagram for the best-fitting common pathway model



Legend: path diagram depicts factor loadings onto twin 1 (T1) and twin 2 (T2) for hyperactivity-impulsivity (HI), inattention (IA) and emotional lability (EL); F = common latent factor; A = additive genetic component of variance; D = non-additive genetic component; E = non-shared environmental component; C suffix denotes common variance component; S suffix denotes specific variance component; *b* = contrast effect, modelled for males only for EL; α = coefficient of additive genetic relatedness between T1 & T2, set to 1.00 for MZ pairs and 0.5 for DZ pairs; β = coefficient of non-additive genetic relatedness between T1 & T2, set to 1.00 for MZ pairs and 0.25 for DZ pairs; dashed lines denote non-significance; parameter estimates presented in Table 5.6.

Table 5.6 Standardised parameter estimates for the best-fitting common pathway model

	F	HI	IA	EL
A_C^2	0.89 (0.87, 0.91)	-	-	-
E_C^2	0.11 (0.09, 0.13)	-	-	-
F^2	-	0.77 (0.74, 0.79)	0.67 (0.64, 0.69)	0.53 (0.49, 0.55)
A_S^2	-	0.00 (0.00, 0.10)	0.00 (0.00, 0.06)	0.00 (0.00, 0.07)
D_S^2	-	0.14 (0.04, 0.17)	0.18 (0.12, 0.21)	0.25 (0.17, 0.28)
E_S^2	-	0.09 (0.07, 0.11)	0.16 (0.13, 0.18)	0.23 (0.20, 0.26)
b	-	-0.10 (-0.13, -0.07)	-0.10 (-0.13, -0.08)	-0.06 (-0.10, -0.02)

Note: F = latent factor; HI = hyperactivity-impulsivity; IA = inattention; E = emotional lability; A_C^2 = standardised additive genetic component for latent factor; D_C^2 = standardised non-additive genetic component for latent factor; E_C^2 = standardised non-shared environmental component for latent factor; F^2 = latent factor loading for each phenotype; A_S^2 = specific additive genetic component for each phenotype; E_S^2= specific non-shared environmental component for each phenotype; *b* = contrast effect; 95% confidence intervals in parentheses.

Table 5.7 Percentage of variance due to common vs. specific genetic/ environmental effects

	HI	IA	EL
Common A	69%	60%	47%
Common E	8%	7%	6%
Specific A	0%	0%	0%
Specific D	14%	18%	25%
Specific E	9%	16%	23%

Note: percentage of total variance explained in hyperactivity-impulsivity (HI), inattention (IA) and emotional lability (EL), calculated using values in Table 5.6; percentage due to common effects calculated as the standardised common factor loading multiplied by the standardised common parameter estimate, multiplied by 100 (i.e. Common A = [F^2 * A_C^2] * 100); proportion due to specific effects calculated as standardised specific parameter estimate multiplied by 100 (i.e. Specific E = E_S^2 * 100).

5.4.5 *Ad-hoc* modelling: bivariate analyses

After controlling for hyperactivity-impulsivity, the genetic correlation (r_A) between inattention and emotional lability was modest but significant (r_A = 0.25), indicating a unique genetic association. Similarly, there was a significant genetic correlation between hyperactivity-impulsivity and emotional lability (r_A = 0.43) after controlling for inattention. Non-overlapping confidence intervals indicated that the genetic correlation was significantly larger for emotional lability with hyperactivity-impulsivity than with inattention (see Tables 5.8 to 5.9).

Table 5.8 Fit statistics for the *ad-hoc* bivariate modelling comparing hyperactivity-impulsivity and inattention

Model (parameters)	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
EL with IA							
Saturated	16161.33	7648	865.33	-20829.08	-	-	-
CFS (A, D, E, r_A, r_D, r_E, *b*)	16204.54	7663	878.54	-20864.18	-	-	-
CFS (A, E, r_A, r_E, *b*)	**16220.34**	**7668**	**884.34**	**-20875.18**	**20.77**	**16**	**> .05**
EL with HI							
Saturated	15333.82	7648	37.82	-21242.84	-	-	-
CFS (A, D, E, r_A, r_D, r_E, *b*)	15370.50	7663	44.50	-21281.20	-	-	-
CFS (A, E, r_A, r_E, *b*)	**15378.05**	**7668**	**42.05**	**-21296.32**	**20.09**	**16**	**> .05**

Note: fit statistics for bivariate saturated model and bivariate correlated factors solution (CFS) of the Cholesky decomposition; upper section for emotional lability (EL) with inattention (IA) after controlling for hyperactivity-impulsivity (HI); lower section for EL with HI after controlling for IA; -2LL = log likelihood statistic; df = degrees of freedom; AIC = Akaike's Information Criterion; BIC = Bayesian Information Criterion; χ^2 = likelihood ratio test for difference between full and restricted models; Δdf = difference in degrees of freedom for LRT; *p* = significance of LRT; best-fitting model denoted in **bold**.

Table 5.9 Parameter estimates for the *ad-hoc* bivariate modelling comparing hyperactivity-impulsivity and inattention

Model	r_A	r_E	r_P	A^2	E^2	*b*
EL with IA	0.25 (0.20, 0.31)	0.09 (0.02, 0.16)	0.21 (0.17, 0.24)	-	-	-
IA	-	-	-	0.68 (0.72, 0.73)	0.32 (0.27, 0.38)	-0.20 (-0.24, -0.16)
EL	-	-	-	0.72 (0.68, 0.76)	0.28 (0.24, 0.32)	-0.08 (-0.13, -0.04)
EL with HI	0.43 (0.38, 0.48)	0.05 (-0.02, 0.12)	0.33 (0.29, 0.36)	-	-	-
HI	-	-	-	0.73 (0.68, 0.77)	0.27 (0.23, 0.32)	-0.19 (-0.22, -0.15)
EL	-	-	-	0.73 (0.69, 0.76)	0.27 (0.24, 0.31)	-0.09 (-0.13, -0.05)

Note: parameter estimates for correlated factors solution of Cholesky decomposition; upper section for emotional lability (EL) with inattention (IA), after controlling for hyperactivity-impulsivity (HI); lower section for EL with HI, after controlling for IA; r_A, additive genetic correlation; r_E, non-shared environmental correlation; r_P, phenotypic correlation; A^2, standardised additive genetic influences; E^2, standardised non-shared environmental influences; *b,* contrast effect, included for boys only for *EL in both sets of models*.

Table 5.10 Fit statistics for the *ad-hoc* age-stratified analyses – younger cohort

Model	Parameters	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated	-	11361.42	5223	915.42	-12025.95	-	-	-
CFS	A, D, E, r_A, r_D, r_E, *b*	11426.05	5250	926.05	-12084.27	-	-	-
IP	A_C, D_C, E_C, A_S, D_S, E_S, *b*	11426.44	5250	926.44	-12084.08	-	-	-
CP	A_C, D_C, E_C, A_S, D_S, E_S, *b*	11430.88	5254	922.88	-12095.42	-	-	-
CP	A_C, E_C, A_S, D_S, E_S, *b*	11430.88	5255	920.88	-12098.81	0.00	1	1.00
CP	A_C, D_C, E_C, A_S, E_S, *b*	11464.37	5257	950.37	-12088.84	33.49	3	<.001
CP	A_C, E_C, A_S, E_S, *b*	11464.37	5258	948.37	-12092.23	33.49	4	<.001
CP	A_C, D_C, E_C, A_S, D_S, E_S, *b**	11456.98	5255	946.98	-12085.75	26.10	1	<.001
CP	A_C, D_C, E_C, A_S, D_S, E_S, *b***	11474.97	5255	964.97	-12076.76	44.10	1	<.001
CP	A_C, D_C, E_C, A_S, D_S, E_S, *b****	11432.25	5255	922.25	-12098.12	1.38	1	0.24
CP	A_C, D_C, E_C, A_S, D_S, E_S	11481.19	5257	967.19	-12080.43	50.31	3	<.001
CP	A_C, E_C, A_S, D_S, E_S	11491.38	5258	975.38	-12078.72	60.50	4	<.001
CP	A_C, D_C, E_C, A_S, E_S	11523.91	5260	1003.91	-12069.24	93.03	6	<.001
CP	A_C, E_C, A_S, E_S	11537.02	5261	1015.02	-12066.08	106.14	7	<.001
CP	**A_C, E_C, A_S, D_S, E_S, *b***	**11436.76**	**5256**	**924.76**	**-12099.26**	**5.88**	**2**	**0.053**

Note: -2LL = log likelihood statistic; df = degrees of freedom; AIC = Akaike's Information Criterion; BIC = Bayesian Information Criterion; χ^2 = likelihood ratio test (LRT) for difference between full and restricted models; Δdf = difference in degrees of freedom for LRT; *p* = significance of LRT; CFS = correlated factors solution of the Cholesky decomposition; IP = independent pathway model; CP = common pathway model; all models constrained male variances to be a scalar multiple of female variances for parent, teacher and self ratings; for EL contrast effects (*b*) were included for males only; *dropped the contrast effect for HI; **dropped the contrast effect for IA; ***dropped the contrast effect for EL; best-fitting model denoted in **bold**, in which *b* was dropped for EL but not HI or IA, in addition to dropping common D.

Table 5.11 Fit statistics for the *ad-hoc* age-stratified analyses – older cohort

Model	Parameters	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated	-	13227.39	6183	861.39	-14862.88	-	-	-
CFS	A, D, E, r_A, r_D, r_E, *b*	13281.78	6210	861.78	-14929.47	-	-	-
IP	A_C, D_C, E_C, A_S, D_S, E_S, *b*	13291.44	6214	863.44	-14938.53	-	-	-
CP	A_C, E_C, A_S, D_S, E_S, *b*	13291.44	6215	861.44	-14942.01	-	-	-
CP	**A_C, D_C, E_C, A_S, E_S, *b***	**13312.77**	**6217**	**878.77**	**-14938.29**	**0.00**	**1**	**1.00**
CP	A_C, E_C, A_S, E_S, *b*	13312.77	6218	876.77	-14941.76	21.33	3	<.001
CP	A_C, D_C, E_C, A_S, D_S, E_S, *b**	13325.34	6215	895.34	-14925.06	21.33	4	<.001
CP	A_C, D_C, E_C, A_S, D_S, E_S, *b***	13309.42	6215	879.42	-14933.02	33.90	1	<.001
CP	A_C, D_C, E_C, A_S, D_S, E_S, *b****	13300.05	6215	870.05	-14937.71	17.98	1	<.001
CP	A_C, D_C, E_C, A_S, D_S, E_S	13336.69	6217	902.69	-14926.33	8.60	1	<.01
CP	A_C, E_C, A_S, D_S, E_S	13338.94	6218	902.94	-14928.68	45.25	3	<.001
CP	A_C, D_C, E_C, A_S, E_S	13372.12	6220	932.12	-14919.04	47.50	4	<.001
CP	A_C, E_C, A_S, E_S	13375.98	6221	933.98	-14920.58	80.68	6	<.001
CP	A_C, D_C, E_C, A_S, D_S, E_S, *b*	13281.78	6210	861.78	-14929.47	84.53	7	<.001

Note: -2LL = log likelihood statistic; df = degrees of freedom; AIC = Akaike's Information Criterion; BIC = Bayesian Information Criterion; χ^2 = likelihood ratio test (LRT) for difference between full and restricted models; Δdf = difference in degrees of freedom for LRT; *p* = significance of LRT; CFS = correlated factors solution of the Cholesky decomposition; IP = independent pathway model; CP = common pathway model; all models constrained male variances to be a scalar multiple of female variances for parent, teacher and self ratings; for EL contrast effects (*b*) were included for males only; *dropped the contrast effect for HI; **dropped the contrast effect for IA; ***dropped the contrast effect for EL; best-fitting model denoted in **bold**.

Table 5.12 Standardised parameter estimates for the best-fitting common pathway model

	F	HI	IA	EL
Younger				
A_C^2	0.87 (0.83, 0.90)	-	-	-
E_C^2	0.13 (0.10, 0.17)	-	-	-
F^2	-	0.77 (0.72, 0.83)	0.66 (0.62, 0.71)	0.46 (0.42, 0.50)
A_S^2	-	0.00 (0.00, 0.15)	0.00 (0.00, 0.09)	0.00 (0.00, 0.12)
D_S^2	-	0.14 (0.00, 0.18)	0.20 (0.11, 0.25)	0.32 (0.19, 0.37)
E_S^2	-	0.09 (0.06, 0.12)	0.14 (0.11, 0.18)	0.21 (0.17, 0.25)
b	-	-0.10 (-0.15, -0.07)	-0.14 (-0.17, -0.10)	-
Older				
A_C^2	0.91 (0.89, 0.93)	-	-	-
E_C^2	0.09 (0.07, 0.11)	-	-	-
F^2	-	0.76 (0.72, 0.79)	0.67 (0.62, 0.71)	0.58 (0.53, 0.61)
A_S^2	-	0.00 (0.00, 0.17)	0.00 (0.00, 0.13)	0.00 (0.00, 0.11)
D_S^2	-	0.14 (0.00, 0.18)	0.16 (0.04, 0.20)	0.21 (0.09, 0.25)
E_S^2	-	0.09 (0.07, 0.12)	0.17 (0.14, 0.22)	0.22 (0.18, 0.26)
b	-	-0.10 (-0.15, -0.07)	-0.08 (-0.12, -0.04)	-0.08 (-0.13, -0.03)

Note: upper section gives parameter estimates for younger cohort, lower section for older cohort; F = latent factor; HI = hyperactivity-impulsivity; IA = inattention; EL = emotional lability; A_C^2 = standardised additive genetic component for latent factor; D_C^2 = standardised non-additive genetic component for latent factor; E_C^2 = standardised non-shared environmental component for latent factor; F^2 = latent factor loading for each phenotype; A_S^2 = specific additive genetic component for each phenotype; E_S^2= specific non-shared environmental component for each phenotype; *b* = contrast effect; 95% confidence intervals in parentheses.

5.4.6 *Ad-hoc* modelling: age-stratified analyses

Consistent with results for the whole sample, a common pathway model provided the best fit to the data in both age-stratified cohorts (Tables 5.10 and 5.11). Parameter estimates are presented in Table 5.12. Non-overlapping confidence intervals indicated that the factor loading (F^2) for *EL* was significantly stronger in the older than younger cohort, and that the contrast effect for *EL* was non-significant in the younger cohort. There were no other significant differences between cohorts.

5.5 DISCUSSION

This study used a multivariate twin design to investigate the aetiological relationship between symptom dimensions of hyperactivity-impulsivity, inattention and emotional lability in a large, community sample of children and adolescents. The main finding was that all three dimensions were significantly related and that phenotypic co-variation was primarily due to common genetic influences. A common pathway model provided the best empirical fit to the data, suggesting that symptoms of hyperactivity-impulsivity, inattention and emotional lability contributed to a highly heritable latent factor, which might be viewed as representing a broader ADHD phenotype than exists in current taxonomy.

The findings advance existing literature by demonstrating a clear aetiological link between emotional lability and ADHD that is primarily due to genetic and not environmental factors. Previous research has established a strong genetic association between the dimensions of hyperactivity-impulsivity and inattention (Greven et al., 2011a, Larsson et al., 2013, McLoughlin et al., 2007), which the present study suggests is also largely shared with emotional lability. The common genetic influences are consistent with recent concepts arising from child and adult ADHD literature proposing that emotional lability reflects a core component of ADHD, as evidenced by the strong phenotypic associations in clinical populations (Barkley, 2010, Skirrow et al., 2009) and the marked co-variation of the three symptom domains during the treatment response (Marchant et al., 2011a, Marchant et al., 2011b, Reimherr et al., 2005b, Reimherr et al., 2007, Rosler et al., 2010). The present findings also explain familial co-segregation of ADHD and emotional lability (Biederman et al., 2012d, Surman et al., 2011), indicating that it primarily reflects common genetic effects.

However, the present study also identified unique non-additive genetic and non-shared environmental influences for symptoms of hyperactivity-impulsivity, inattention and emotional lability, indicating that the aetiological overlap between dimensions was not absolute. Unique aetiological influences are consistent with the results of one recent neuropsychological study, which found that cognitive performance deficits linked with ADHD, such as inhibitory deficits

and reaction time variability, were not directly associated with emotional lability (Banaschewski et al., 2012). Therefore, the common genetic influences found in the present study may not reflect common neurobiological pathways from genes to behaviour and could instead reflect pleiotropic genetic effects. Shared treatment effects and co-variation of symptoms during the treatment response reported in the literature suggest that such divergence might occur downstream of common neurobiological substrates involving dopamine regulation. Further research is required, from genetic and neuropsychological perspectives, to test this hypothesis.

That emotional lability was associated with both inattention and hyperactivity-impulsivity diverges somewhat from previous research, including a large clinical study of children and adolescents with ADHD and their siblings (Sobanski et al., 2010). This study identified an association with hyperactivity-impulsivity only and is of particular interest as it included a sample of the same age-range as reported on here. One possible explanation for this difference is ascertainment bias, since the clinical sample included young people with combined type ADHD selected for impairment, in addition to high levels of hyperactive-impulsive and inattentive symptoms. This is particularly relevant since perceived impairment leading to clinical referrals may reflect greater severity of externalising behaviours, including more severe ratings of hyperactive-impulsive and emotional lability symptoms. Another, related explanation is that the use of parent-only ratings of behaviour in this study may have influenced the pattern of results, since the clinical study used composite ratings from parents and teachers. However, since the phenotypic and *ad-hoc* genetic analyses in the present study demonstrated a significantly stronger association of emotional lability with hyperactivity-impulsivity than inattention, it can be concluded that the pattern of findings differs only in degree.

Ad-hoc analyses also indicated an age effect, with emotional lability more strongly related to the latent ADHD factor in older than younger twins. This suggests greater sharing of genetic influences between hyperactivity-impulsivity, inattention and emotional lability in older individuals, and the emergence of emotional lability as more closely aligned to the core ADHD phenotype. One explanation is that emotional lability in childhood may be

qualitatively different from emotional lability in adolescence. For example, emotional lability in childhood could arise for a number of reasons besides ADHD; however as these heterogeneous symptoms taper off during development, what is left might be a chronic state of emotional lability, primarily related to ADHD. The age-stratified analyses took a pragmatic approach and the conclusions drawn here are speculative; further research is thus required to examine developmental-genetic associations of emotional lability with ADHD.

One inference from the main results is that emotional lability may form an integral component of a broader ADHD construct. This is supported by converging evidence from familial and therapeutic research. However, many other cognitive and behavioural traits share genetic risk factors with ADHD and would not be perceived in this way, including autism (Ronald et al., 2008), dyslexia (Greven et al., 2011b) and depression (Cole et al., 2009). Furthermore, emotional lability is a common trait seen to occur across conditions (Kring and Sloan, 2010) and is therefore not specific to ADHD. Alternative explanations should therefore also be considered, one of which is that the common genetic liability for ADHD and emotional lability reflects a more general latent construct that cuts across a range of disorders. This is consistent with genetic studies linking ADHD to other conditions characterised by irritability and volatile mood, such as oppositional defiant disorder and bipolar disorder (see section 1.8.1), and with the introduction of disruptive mood dysregulation disorder as a unique diagnostic entity in DSM5 (American Psychiatric Association, 2013). It is therefore important for future research to examine the validity of emotional lability as a transdiagnostic construct.

Several sets of limitations should be considered when interpreting the results of this study. First, the definition of emotional lability has differed throughout the literature, despite the similar face validity of items used. Therefore it is unclear how well findings will replicate in studies that do not use the same measure of emotional lability symptoms reported on here. It is also unclear how the construct of emotional lability differs from other phenotypes that feature similar symptoms. The similarity in item content between ODD and emotional lability is particularly relevant in this study, since ODD symptoms are strongly genetically related to hyperactivity-impulsivity (Wood et al., 2009a). Therefore the present

analyses might simply index a relationship between ADHD and an irritable component of ODD. Because data were not available on ODD in this sample, this limitation could not be addressed via additional analyses. However, the present study builds on existing literature by focussing on a purely irritable/ emotionally labile symptom dimension in relation to ADHD and by demonstrating association with hyperactivity-impulsivity and inattention. This set of limitations highlights the need for a consensus definition of emotional lability from the wider scientific community and for further psychopathological research.

Another set of limitations relates to the methodological strategies employed in this research. First, ADHD and emotional lability were assessed as continuous symptom dimensions in a community twin sample, meaning that results may not generalise to clinical populations. Second, this study used restricted twin models (i.e. those with parameters dropped). Such models are more easily interpreted than full models, although by dropping non-additive genetic parameters the estimates of total genetic influence may have been inflated. True estimates therefore lie somewhere within the 95% confidence intervals reported and replication is required. Third, the present analyses were based on parental ratings of ADHD that were subject to contrast effects, presumed to be a form of rater bias (Simonoff et al., 1998). Contrast effects have been found previously in this sample, but for inattentive ADHD symptoms only (Thapar et al., 2000). One possible explanation for this difference in results is that the previous study examined symptoms of hyperactivity and impulsivity separately, whereas the present study concatenates these symptoms into a single dimension. One solution for future analyses is to use multiple informant ratings of ADHD to form latent constructs that better capture a pervasive view of behaviours (see chapter 3).

Despite these limitations, the results demonstrate common genetic influences for hyperactivity-impulsivity, inattention and emotional lability in children and adolescents. These findings have important implications. For clinical practice, these findings support consideration and evaluation of emotional lability as a related feature of ADHD, alongside the core items listed in DSM-IV and DSM-5. Therefore, ADHD should be considered as a differential diagnosis in individuals presenting with labile, volatile emotions, while emotional lability symptoms

should form a key treatment target for both pharmacological and non-pharmacological interventions. At the level of empirical research, further work is now required to refine the ADHD phenotype and to establish the neurobiological processes arising from the underlying genetic influences. Such research will allow further evaluation of the hypothesis that emotional lability symptoms may reflect an integral feature of ADHD.

6. ADHD, EMOTIONAL LABILITY AND COGNITIVE PERFORMANCE: TESTING FOR PHENOTYPIC AND GENETIC MEDIATION

6.1 OVERVIEW

The previous chapter linked ADHD to emotional lability at both the phenotypic and aetiological levels, yet the neurocognitive basis of this association remains poorly understood. The aim of chapter 6 was to examine the association of ADHD and emotional lability symptoms with cognitive performance using a genetically-sensitive design. Participants were 668 child twin pairs aged 7 to 9 years. Symptoms of hyperactivity-impulsivity, inattention and emotional lability were assessed using the Long Version of Conners' Rating Scale, completed by parents and teachers. Cognitive performance was assessed using laboratory-based computerised tasks. Regression analyses indicated that a range of cognitive performance measures were weakly but significantly associated with emotional lability, however after controlling for ADHD symptoms of hyperactivity-impulsivity and inattention these associations were attenuated to a non-significant level, indicating possible mediation. Structural equation modelling confirmed that the phenotypic association between emotional lability and reaction time variability was mediated via the symptoms of ADHD, while genetic models indicated that this covariance was primarily due to a common genetic liability. These findings suggest that there is no direct relationship of emotional lability with the cognitive performance deficits implicated in ADHD.

6.2 INTRODUCTION

The results presented in chapter 5 demonstrated a shared aetiology, primarily genetic in origin, for the symptoms of ADHD and emotional lability among a community sample of child and adolescent twins. This builds on a growing body of clinical evidence arguing that emotional lability is a primary deficit in ADHD (for reviews see Barkley, 2010, Corbisiero et al., 2013, Retz et al., 2012, Skirrow et al., 2009). Yet the mechanisms linking common sets of genes to

emotional lability and ADHD remain unclear. Common genetic influences could indicate that the same neurocognitive substrates underlie ADHD and emotional lability, with the same neurobiological pathways from genes to behaviour. Alternatively, common genetic influences could indicate pleiotropy, whereby ADHD and emotional lability have the same underlying genetic liability but distinct pathways from genes to behaviour. One way to investigate these competing hypotheses is to examine the relationship of ADHD and emotional lability symptoms with cognitive performance in a genetically-sensitive design.

Family and twin research has demonstrated aetiological associations of ADHD with deficits in cognitive performance. Many findings arise from two parallel studies: the International Multicentre ADHD Genetics (IMAGE) project, a family study of ADHD probands and siblings; and the Study of Activity and Impulsivity Levels in children (SAIL), a population-based twin cohort. These studies have consistently linked ADHD to slower mean reaction time (MRT), greater reaction time variability (RTV) and a greater number of commission errors (CE) on cognitive performance tasks (Andreou et al., 2007, Kuntsi et al., in 2013, Kuntsi et al., 2010, Kuntsi et al., 2009, Uebel et al., 2010a). Familial analyses indicate moderate-to-strong familial correlations (r_F) of total ADHD symptoms with MRT (r_F = 0.61), RTV (r_F = 0.74) and CE (r_F = 0.45), and a separation of reaction time (RT, i.e. MRT/RTV) and CE into distinct familial factors (Kuntsi et al., 2010). Twin analyses have estimated moderate genetic correlations (r_A) for the symptoms of hyperactivity-impulsivity versus inattention with MRT (r_A = 0.19 vs. 0.56), RTV (r_A = 0.31 vs. 0.64) and CE (r_A = 0.17 vs. 0.11), and have confirmed the separation of RT from CE (Kuntsi et al., in 2013).

Research therefore identifies a common aetiology for ADHD and cognitive performance deficits, but with a separation of regulatory (MRT, RTV) and inhibitory (CE) processes. This is consistent with major cognitive theories of ADHD, which propose that top-down inhibitory deficits and bottom-up arousal dysregulation characterise distinct pathways to behaviour (Barkley, 1997, Halperin et al., 2008, Kuntsi and Klein, 2012, Nigg et al., 2005, Sergeant, 2005). These theories have recently been expanded to form working hypotheses regarding the emergence of emotional lability.

First, it is proposed that emotional lability could arise as a result of deficient state regulation in ADHD (Skirrow et al., 2009). If this is the case then studies should find evidence of association between emotional lability and MRT/RTV. Second, it is proposed that executive dysfunction, including poor inhibitory control, leads to dysregulation of behaviour and emotion in ADHD (Barkley, 2010). If this is the case then studies should find evidence of association between emotional lability and measures of inhibition (CE) and/or other executive functions (e.g. sustained attention, working memory). Third, it is proposed that individuals with ADHD are delay averse, reacting emotionally and with frustration in response to delay (Sonuga-Barke, 2005). If this is the case then there should be association of emotional lability with measures of delay aversion, including actions aimed at minimising or reducing delay in salient conditions (e.g. choice impulsivity, see Paloyelis et al., 2009).

Two recent studies have examined the associations between ADHD, emotional lability and cognitive performance. The first study, conducted in IMAGE, found low-to-modest associations of emotional lability with measures of MRT (standardised regression coefficient [SRC] = 0.36), RTV (SRC = 0.30) and CE (SRC = 0.19), in addition to associations with sustained attention (omission errors, SRC = 0.28), working memory (digit span backwards, SRC = -0.15) and choice impulsivity (SRC = 0.11) (Banaschewski et al., 2012). There was no significant association with delay aversion. However, these associations were attenuated to a non-significant level after controlling for the symptoms of ADHD. The second study found that adults with ADHD and deficient emotional self-regulation (DESR; i.e. severe symptoms of emotional lability) did not differ significantly from adults with ADHD without DESR across measures of executive functioning (Surman et al., 2013). Overall, these studies find that while emotional lability is associated with some of the same cognitive performance deficits as ADHD, there is no indication that these deficits lead directly to the symptoms of emotional lability. Instead, the association appears to be indirect and possibly mediated via the symptoms of ADHD themselves (Banaschewski et al., 2012).

Mediation occurs when a third variable explains some of the association between two other variables (Baron and Kenny, 1986). This scenario does not

preclude the existence of shared genetic influences across the dimensions of ADHD, emotional lability and cognitive performance; however, the nature of any shared genetic effects requires careful consideration (Kendler and Neale, 2010). On the one hand, cognitive performance deficits may have a causal influence on the development of ADHD, which in turn may have a causal influence on emotional lability. The implication is that the genetic influences on cognitive performance might have an indirect effect on emotional lability, mediated via the symptoms of ADHD. The alternative hypothesis is one of a common liability, where the same genetic effects have a pleiotropic influence across dimensions of ADHD, emotional lability and cognitive performance, but without a truly mediated effect. Neither hypothesis has yet been examined.

In order to test these competing models, the present study utilised twin data from SAIL to assess the phenotypic and genetic associations of ADHD and emotional lability with cognitive performance during middle childhood. As a first step, a replication of the IMAGE findings reported by Banaschewski et al. (2012) was undertaken. As a second step, the associations between ADHD, EL and cognitive performance were examined using a genetically-sensitive design. Structural equation modelling tested for a mediated phenotypic association between RTV, ADHD and EL. A genetic model, first described by Kendler et al. (1993), was then fit to compare the effects of a mediated (causal) versus a common (correlated) liability. The ADHD dimensions of hyperactivity-impulsivity and inattention were modelled separately, in line with prior evidence of a stronger association of emotional lability with hyperactive-impulsive symptoms (see chapter 5), and a differential association of cognitive performance deficits with hyperactivity-impulsivity and inattention (Kuntsi et al., in 2013).

6.3 METHOD

6.3.1 Sample and measures

The sample was from the Study of Activity and Impulsivity Levels in children (SAIL). Full details on the sample and all measures used are provided in section 2.2.4. The present analyses focused on a total of 1,312 children from 668 twin pairs: 124 monozygotic males (MZM; no incomplete pairs), 96

monozygotic females (MFZ; 2 incomplete pairs), 136 dizygotic males (DZM; 3 incomplete pairs), 92 dizygotic females (5 incomplete pairs) and 220 dizygotic opposite-sex pairs (DZO; 14 incomplete pairs). The mean age of participating children was 8.83 years (SD = 0.67).

ADHD and emotional lability symptoms were assessed using the Long Version of Conners' Parent Rating Scale (Conners et al., 1998a) and the Long Version of Conners' Teacher Rating Scale (Conners et al., 1998b). Parent and teacher responses were summed to create composite scores for hyperactivity-impulsivity (9 items plus 9 items), inattention (9 items plus 9 items) and emotional lability (3 parent-rated items plus 4 teacher-rated items). The separation of emotional lability from ADHD has been documented in prior factor analytic research (Chen, unpublished data, Parker et al., 1996, Westerlund et al., 2009).

Measures of cognitive performance were derived in several ways. The vocabulary, similarities, picture completion and block design subtests from the Wechsler Intelligence Scale for Children, Third Edition (WISC-III) (Wechsler, 1991) were used to assess IQ, with digit span forwards (DSF) and backwards (DSB) used to assess short-term and working memory. Performance on the Go/No-go task (Borger and van der Meere, 2000, Kuntsi et al., 2005a, van der Meere et al., 1995) and the Fast task (Andreou et al., 2007, Kuntsi et al., 2005a, Kuntsi et al., 2006) were used to derive composite measures of mean reaction time (MRT) and reaction time variability (RTV), while the Go/No-go task was additionally used to assess commission errors (CE). Performance on the Maudsley Index of Delay Aversion (Kuntsi et al., 2001a, Kuntsi et al., 2006, Paloyelis et al., 2009) was used to measure choice impulsivity (CI).

6.3.2 Statistical analyses

Preliminary analyses were conducted using Stata version 10.1 (StataCorp., 2007). Robust regressions examined associations between the cognitive performance variables and emotional lability before and after controlling for hyperactivity-impulsivity and inattention. Prior to regressing emotional lability on cognitive performance, all variables were first regressed on age and sex, while

all cognitive variables (apart from IQ) were additionally regressed on IQ. Residuals were taken forward in analyses so as to remove potential confounding effects. Residuals for variables apart from CE and IQ were then transformed using the Stata command *lnskew0*, ensuring that all data distributions were within the normal range (skewness = 0±1 and kurtosis = 3±1). Finally, all variables were standardised to a mean of zero and standard deviation of 1 so that standardised regression coefficients could be obtained.

Structural equation modelling was conducted in Mx (Neale et al., 2006) using transformed data regressed on age/sex/IQ, in line with standard twin modelling procedures (McGue and Bouchard Jr, 1984). Univariate sex-limitation models first decomposed phenotypic variances in into genetic and environmental components while also testing for aetiological sex differences (see section 2.3.6). Models parameterised additive genetic (A) and non-shared environmental (E) components of variance, in addition to either shared-environmental (C) or non-additive genetic (D) components depending on the observed twin correlations.

To test for mediation, the criteria from Baron and Kenny (1986) were applied:

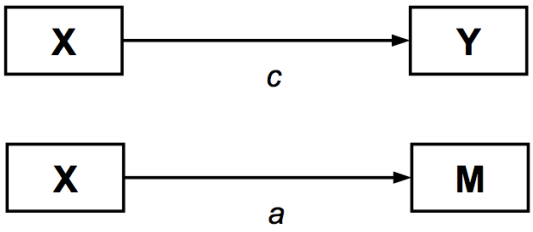
1. Demonstrate a significant bivariate association between the independent variable (X) and the dependent variable (Y) (tests path *c* in Figure 6.1).

2. Demonstrate a significant bivariate association between X and the putative mediator variable (M) (equivalent to a test of path *a* in Figure 6.1).

3. Demonstrate a significant association between M and Y, while controlling the effect of X on Y (equivalent to a test of path *b* in Figure 6.2).

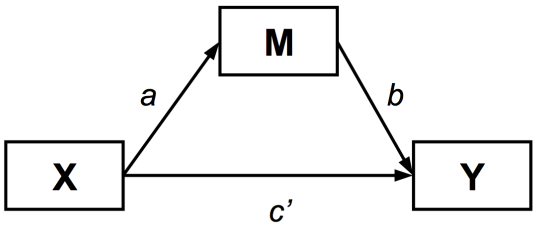
4. Demonstrate an attenuated association between X and Y while controlling for the effects of M on Y (equivalent to a test of path *c'* in Figure 6.2). If path *c'* is attenuated but still significant then this is evidence of partial mediation; if path *c'* is no longer significant then this is evidence of complete mediation.

Figure 6.1. Bivariate association paths for phenotypic mediation models



Legend: Path *c* represents the bivariate phenotypic association between the predictor variable (X) and the criterion variable (Y); path *a* represents the bivariate phenotypic association between the predictor variable (X) and the mediator variable (M); paths *a* and *c* must be significant for mediation to occur.

Figure 6.2. The full phenotypic mediation model



Legend: A three-variable phenotypic mediation model. Path *a* represents the association between the predictor variable (X) and the mediator variable (M); path *b* represents the association between M and the criterion variable (Y); path *c'* represents the association between X and Y while controlling for M. In structural equation models, paths *a, b & c'* can be estimated simultaneously and can be dropped in turn to assess their significance; paths *a, b & c'* can be estimated separately for males and females or can be equated across sex.

A structural equation model for phenotypic mediation was created based on the diagram in Figure 6.2 (Iacobucci, 2008). In this model, X accounted for a proportion of the total variance in M and Y via paths *a* and *c'*. M additionally accounted for a proportion of the total variance in Y via path *b*. The full model allowed paths *a*, *b* and *c'* to differ between males and females and was compared to a restricted model with path estimates equated across sex. To test their significance, paths *a*, *b*, and *c'* were dropped in sequence and the changes in model fit examined using likelihood ratio tests (LRTs).

A genetic mediation model, adapted from Kendler *et al*. (1993), was then used to decompose phenotypic covariation into genetic, environmental and mediation components. The model is described in detail in section 2.3.7 (see figure 2.8). Common genetic (A_C) and non-shared environmental (E_C) factors represented the extent to which phenotypic covariation was due to a common liability.

Specific genetic (A_S) and non-shared environmental (E_S) factors were then estimated separately for X, M and Y. These reflected the unique liability for each variable. The model specified mediation paths *a* and *b* but not *c'*, based on the assumption of no direct association between X and Y. The mediation paths therefore represented the extent to which covariation was due to mediated effects, which could be decomposed into genetic and environmental components to reflect a mediated liability. The significance of the common versus mediated liability was assessed by dropping parameters and examining the change in fit using LRTs.

6.4 RESULTS

6.4.1 Descriptive statistics

Descriptive statistics for the raw variables are presented in Table 6.1. Tests of mean differences were performed in Stata using robust regressions to control for dependence in the observations from twin pairs (Williams, 2000). Males scored significantly higher than females for hyperactivity-impulsivity (t = 8.43, p < .001), inattention (t = 9.26, p < .001), emotional lability (t = 2.60, p < .05), MRT (t = 3.76, p <.001), CE (t = 10.48, p = <.001) and IQ (t = 2.51, p < .05). Males scored significantly lower for DSB (t = -2.10, p < .05) and CI (t = -3.07, p < .01). There were no significant differences for RTV (t = -0.11, p = 0.91) or DSF (t = -1.82, p = 0.07).

Levene's test was used to assess for equality of variances by sex and zygosity for each phenotype. The results (Table 6.2) revealed significantly greater variances among males for hyperactivity-impulsivity, inattention and emotional lability, and significantly greater variances among females for CI. Tests by zygosity revealed significantly greater variances among DZ than MZ twins for inattention and significantly greater variances among MZ twins for CI. These differences were confirmed using the saturated phenotypic model, which indicated a significant deterioration in fit when variances were constrained to be equal across sex or across zygosity for inattention (p < .01, respectively).

Table 6.1 Descriptive statistics for all variables

	Mean (Standard Deviation)				
	All	MZM	MZF	DZM	DZF
HI	9.22 (8.18)	11.06 (8.61)	6.74 (5.89)	11.53 (9.64)	7.32 (6.49)
IA	9.36 (10.14)	12.70 (8.94)	7.79 (6.51)	14.25 (11.14)	9.06 (7.88)
EL	3.04 (2.95)	3.32 (3.19)	2.45 (2.36)	3.25 (3.19)	3.04 (2.82)
MRT	1529.38 (319.53)	1481.79 (322.15)	1587.52 (310.24)	1497.49 (322.12)	1551.48 (314.56)
RTV	627.62 (363.53)	619.06 (350.81)	629.94 (364.15)	631.01 (376.52)	628.04 (359.12)
CE	105.77 (34.40)	116.52 (34.29)	96.42 (31.47)	115.59 (32.90)	95.61 (33.14)
CI	0.31 (0.28)	0.29 (0.30)	0.35 (0.27)	0.28 (0.29)	0.32 (0.27)
DSF	7.78 (1.71)	7.74 (1.80)	7.74 (1.60)	7.64 (1.70)	7.60 (1.73)
DSB	4.45 (1.39)	4.36 (1.42)	4.52 (1.46)	4.37 (1.37)	4.55 (1.35)
IQ	109.34 (14.72)	109.45 (14.64)	107.74 (14.42)	111.17 (15.40)	108.49 (14.09)

Note: statistics reported for raw data; HI = hyperactivity-impulsivity; IA = inattention; EL = emotional lability; MRT = mean reaction time; RTV = reaction time variability; CE = commission errors; CI = choice impulsivity; DSF = digit span forward; DSB = digit span backward; All = statistics reported for whole sample; MZM = monozygotic males; MZF = monozygotic females; DZM = dizygotic same-sex males; DZF = dizygotic same-sex females; consistent with prior studies using this sample, DZ opposite-sex males are grouped with DZM and DZ opposite-sex females are grouped with DZF.

Table 6.2. Levene's test of equality of variances by sex and zygosity

	Tests by sex		Tests by zygosity	
	F	*p*	*F*	*p*
HI	64.48	<.001	3.76	0.05
IA	75.03	<.001	18.44	<.001
EL	10.93	<.001	1.36	0.24
MRT	0.01	0.91	0.40	0.53
RTV	0.44	0.51	0.45	0.50
CE	0.16	0.69	0.18	0.67
CI	17.89	<.001	25.37	<.001
DSF	0.20	0.65	1.00	0.32
DSB	1.41	0.24	2.14	0.14
IQ	1.98	0.15	0.10	0.75

Note: Individual tests for equality of variances; HI = hyperactivity-impulsivity; IA = inattention; EL = emotional lability; MRT = mean reaction time; RTV = reaction time variability; CE = commission errors; CI = choice impulsivity; DSF = digit span forwards; DSB = digit span backwards.

Table 6.3. Regression tests of association between cognitive performance and EL

	Model set 1				Model set 2			
	SRC	SE	*t*	*p*	SRC	SE	*t*	*p*
MRT	0.08	0.03	2.40	<.05	0.02	0.03	.073	0.46
RTV	0.12	0.03	3.82	<.001	0.03	0.03	1.28	0.20
CE	0.07	0.03	2.32	<.05	0.02	0.02	0.89	0.38
CI	0.05	0.03	1.62	0.11	0.01	0.03	0.38	0.71
DSF	-0.07	0.03	-2.15	<.05	-0.03	0.03	-1.26	0.21
DSB	-0.07	0.03	-2.61	<.01	-0.04	0.03	-1.69	0.09
IQ	-0.05	0.03	-1.43	0.15	0.00	0.03	-0.03	0.98

Note: all models used transformed data corrected for age/sex/IQ and standardised, as described above (section 6.3.2); Model set 1 tested for individual associations of each cognitive performance variable with emotional lability (EL); Model set 2 repeated analyses while including hyperactivity-impulsivity and inattention as additional covariates; SRC = unstandardised regression coefficient; SE = standard error of regression coefficient; *t* = *t* test statistic value; *p* = *p* value; MRT = mean reaction time; RTV = reaction time variability; CE = commission errors; CI = choice impulsivity; DSF = digit span forwards; DSB = digit span backwards.

6.4.2 Regressions of emotional lability on cognitive performance

Regression analyses revealed that all cognitive variables apart from CI and IQ were weakly but significantly associated with emotional lability (Model set 1, Table 6.3). However, when controlling for hyperactivity-impulsivity and inattention, all associations between cognitive performance and emotional

lability were attenuated to a non-significant level (Model set 2, Table 6.3). This replicates results reported by Banaschewski et al. (2012) and suggests only an indirect association between cognitive performance and emotional lability. This justifies the use of structural equation modelling to conduct formal tests of mediation. RTV showed the strongest association with emotional lability prior to controlling for ADHD, with a standardised regression coefficient of 0.12. Therefore, only RTV was taken forward for inclusion in subsequent analyses.

6.4.3 Correlations

Twin correlations are presented in the upper section of Table 6.4. Cross-twin within-trait correlations were generally twice as large for MZ than DZ pairs, indicating A influences on hyperactivity-impulsivity, inattention and reaction time variability. For inattention, the lower DZ correlation in relation MZ correlations also suggested possible D influences. Cross-twin cross-trait correlations followed a similar pattern, suggesting mainly shared genetic influences across phenotypes.

The low DZ correlation for inattention is interesting when interpreted in conjunction with the greater phenotypic variance found for DZ than MZ twins (Table 6.2), since this pattern of results is consistent with a contrast effect (Neale and Maes, 2004). However, a contrast effect was not included in the twin models in line with prior conventions within SAIL. This is considered further in the discussion (section 6.5).

Phenotypic correlations are presented in the lower section of Table 6.4. The strongest correlations were for hyperactivity-impulsivity with inattention (r =0.58) and emotional lability (r =0.52). Confidence intervals indicated that these estimates were not significantly different, but that the correlation of inattention with emotional lability (r =0.35) was significantly weaker. For RTV, the strongest correlation was with inattention (r =0.24), followed by hyperactivity-impulsivity (r =0.16) and emotional lability (r =0.12). Confidence intervals indicated that these estimates were not significantly different from one another and that all bivariate correlations with RTV were significant. These correlations indicate the significance of paths *a* and *c* in the mediation models (see Figure 6.1).

Table 6.4. Within-trait cross twin, cross-trait cross-twin and phenotypic correlations

	RTV	HI	IA	EL
RTV	**0.42 (0.32, 0.51)**	**0.13 (0.05, 0.20)**	**0.18 (0.10, 0.25)**	**0.15 (0.07, 0.22)**
	0.23 (0.12, 0.33)			
HI		**0.72 (0.66, 0.77)**	**0.44 (0.37, 0.50)**	**0. 45 (0.39, 0.51)**
	0.05 (-0.03, 0.12)	0.31 (0.21, 0.40)		
IA			**0.62 (0.53, 0.69)**	**0.28 (0.20, 0.35)**
	0.03 (-0.04, 0.10)	0.17 (0.09, 0.25)	0.10 (-0.02, 0.19)	
EL				**0.63 (0.54, 0.69)**
	0.06 (-0.01, 0.13)	0.26 (0.18, 0.34)	0.21 (0.14, 0.29)	0.30 (0.20, 0.39)
RTV	-			
HI	0.16 (0.10, 0.22)	-		
IA	0.24 (0.18, 0.29)	0.58 (0.54, 0.62)	-	
EL	0.12 (0.05, 0.18)	0.52 (0.47, 0.56)	0.35 (0.29 0.40)	**-**

Note: Twin correlations (upper section) reported by zygosity only, in accordance with prior studies using the same sample; within-trait cross-twin correlations on-diagonal, cross-twin cross-trait correlations off-diagonal; **bold text** denotes MZ twin pair correlations, plain text denotes DZ twin pair correlations; pairwise phenotypic correlations are presented in the lower section of this table; 95% confidence intervals in parentheses; fit statistics for saturated model were -2LL = 22561.78, df = 4632, AIC = 13297.78, BIC = -3779.57; RTV = reaction time variability, HI = hyperactivity-impulsivity; IA = inattention; EL = emotional lability.

Table 6.5 Standardised parameter estimates for the best-fitting univariate models

	A^2	D^2	E^2
HI	0.72 (0.66, 0.77)	-	0.28 (0.23, 0.34)
IA	0.00 (0.00, 0.18)	0.62 (0.42, 0.69)	0.38 (0.31, 0.48)
EL	0.63 (0.55, 0.69)	-	0.37 (0.31, 0.45)
RTV	0.42 (0.33, 0.51)	-	0.58 (0.49, 0.67)

Note: A^2 = standardised additive genetic variance component; D^2 = non-additive genetic variance component; E^2 = standardised non-shared environmental variance component; HI = hyperactivity-impulsivity; IA = inattention; EL = emotional lability; RTV = reaction time variability; 95% confidence intervals in parentheses.

6.4.4 Univariate sex-limitation modelling

Full sex-limitation models revealed significant variance (scalar) sex differences for hyperactivity-impulsivity, inattention and emotional lability, controlled for estimating means and variances separately for males and females in all subsequent models. There were no sex differences for RTV. For hyperactivity-impulsivity, emotional lability and RTV the best-fitting models parameterised AE influences; however for inattention there were significant D influences. Model fit statistics are presented in Appendix D and the standardised parameter estimates in Table 6.5. Broad-sense heritabilities were 42% for RTV, 72% for hyperactivity-impulsivity, 62% for inattention and 63% for emotional lability. These are consistent with previous estimates for this sample (Kuntsi et al., in 2013) apart from for emotional lability, reported for the first time here.

6.4.5 Phenotypic mediation modelling

Model fit statistics are presented in Table 6.6. In the first set of models the independent variable (X) was RTV, the mediator variable (M) was hyperactivity-impulsivity and the dependent variable (Y) was emotional lability. The full mediation model (model 1; see Figure 1b) estimated paths *a*, *b* and *c'* for males and females separately, and was compared to restricted models using likelihood ratio χ^2 tests. In model 2, paths *a*, *b* and *c'* were equated across sex. The fit of this model was not significantly worse and consequently all subsequent models equated paths *a*, *b* and *c'* across sex. In model 3 path *c'* could be dropped without a significant deterioration in fit. In models 4 and 5, path *c'* was reinstated while paths *a* and *b* were dropped in turn; however these solutions proved a significantly worse fit. The best-fitting model was therefore model 3, indicating that the phenotypic association between RTV and emotional lability was completely mediated via hyperactivity-impulsivity. In the second set of analyses the mediator variable was switched to inattention and the same series of models fit to the data. Model 3 again provided the best fit, indicating complete mediation of the association between RTV and emotional lability. Residuals (95% confidence intervals) are presented in Figures 6.3 and 6.4.

Figure 6.3. Phenotypic mediation model for reaction time variability (RTV), hyperactivity-impulsivity (HI) and emotional lability (EL)

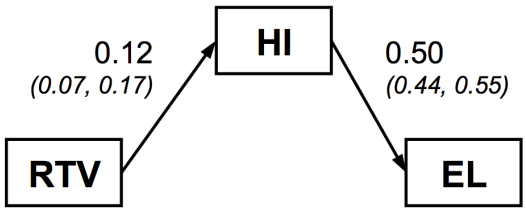


Figure 6.4. Phenotypic mediation model for reaction time variability (RTV), inattention (IA) and emotional lability (EL)

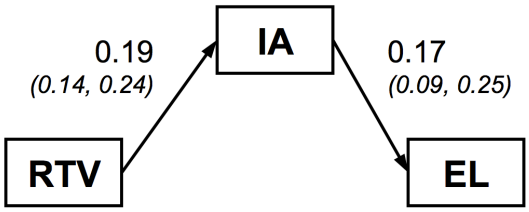


Table 6.6. Fit statistics for the phenotypic mediation models

Model	Parameters	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Phenotypic - Set 1								
1	*a, b, c'* (different across sex)	17527.23	3528	10471.23	-2707.31	-	-	-
2	*a, b, c'* (equated across sex)	17528.51	3531	10466.51	-2716.42	1.29	3	.732
3	**Drop *c'* from model 2**	**17529.07**	**3532**	**10465.07**	**-2719.39**	**1.85**	**4**	**.764**
4	Drop *a* from model 2	17553.07	3532	10489.07	-2707.39	25.85	4	<.001
5	Drop *b* from model 2	17811.13	3532	10747.13	-2578.36	283.90	4	<.001
Phenotypic - Set 2								
1	*a, b, c'* (different across sex)	17789.40	3528	10733.41	-2576.22	-	-	-
2	*a, b, c'* (equated across sex)	17794.87	3531	10732.87	-2583.24	5.46	3	.141
3	**Drop *c'* from model 2**	**17795.20**	**3532**	**10731.20**	**-2586.33**	**5.80**	**4**	**.215**
4	Drop *a* from model 2	17847.06	3532	10783.06	-2560.40	57.65	4	<.001
5	Drop *b* from model 2	17896.21	3532	10832.21	-2535.82	106.80	4	<.001

Note: Fit statistics for phenotypic models with either hyperactivity-impulsivity (Set 1) or inattention (Set 2) included as the mediator variable; -2LL = log likelihood statistic; df = degrees of freedom; AIC = Akaike's Information Criterion; BIC = Bayesian Information Criterion; χ^2 = likelihood ratio test (LRT) for difference between full and restricted models; Δdf = difference in degrees of freedom for LRT; *p* = significance of LRT; best fitting models denoted in **bold**.

Table 6.7. Fit statistics for the genetic mediation models

Model	Parameters	-2LL	DF	AIC	BIC	$\Delta\chi^2$	Δdf	*p*
Genetic - Set 1								
1	A_C, E_C, A_S, E_S, *a, b*	17641.08	3543	10555.08	-2699.15	-	-	-
2	Drop *a* from model 1	17646.03	3544	10558.03	-2699.93	4.94	1	<.05
3	Drop *b* from model 1	17740.76	3544	10652.76	-2652.57	99.68	1	<.001
4	Drop *a & b* from model 1	17744.42	3545	10654.42	-2653.99	107.07	3	<.001
5	Drop A_C from model 1	17656.86	3544	10568.86	-2694.51	15.78	1	<.001
6	**Drop E_C from model 1**	**17641.08**	**3544**	**10553.08**	**-2702.40**	**0.00**	**1**	**1.00**
Genetic - Set 2								
1	A_C, E_C, A_S, E_S, *a, b*	17858.48	3543	10772.47	-2590.46	-	-	-
2	Drop *a* from model 1	17872.01	3544	10784.01	-2586.94	13.55	1	<.001
3	Drop *b* from model 1	17876.29	3544	10788.29	-2584.80	17.82	1	<.001
4	Drop *a & b* from model 1	17879.46	3545	10789.46	-2586.47	20.99	2	<.001
5	Drop A_C from model 1	17879.00	3544	10791.00	-2583.44	20.54	1	<.001
6	**Drop E_C from model 1**	**17858.48**	**3544**	**10770.47**	**-2593.71**	**0.00**	**1**	**1.00**

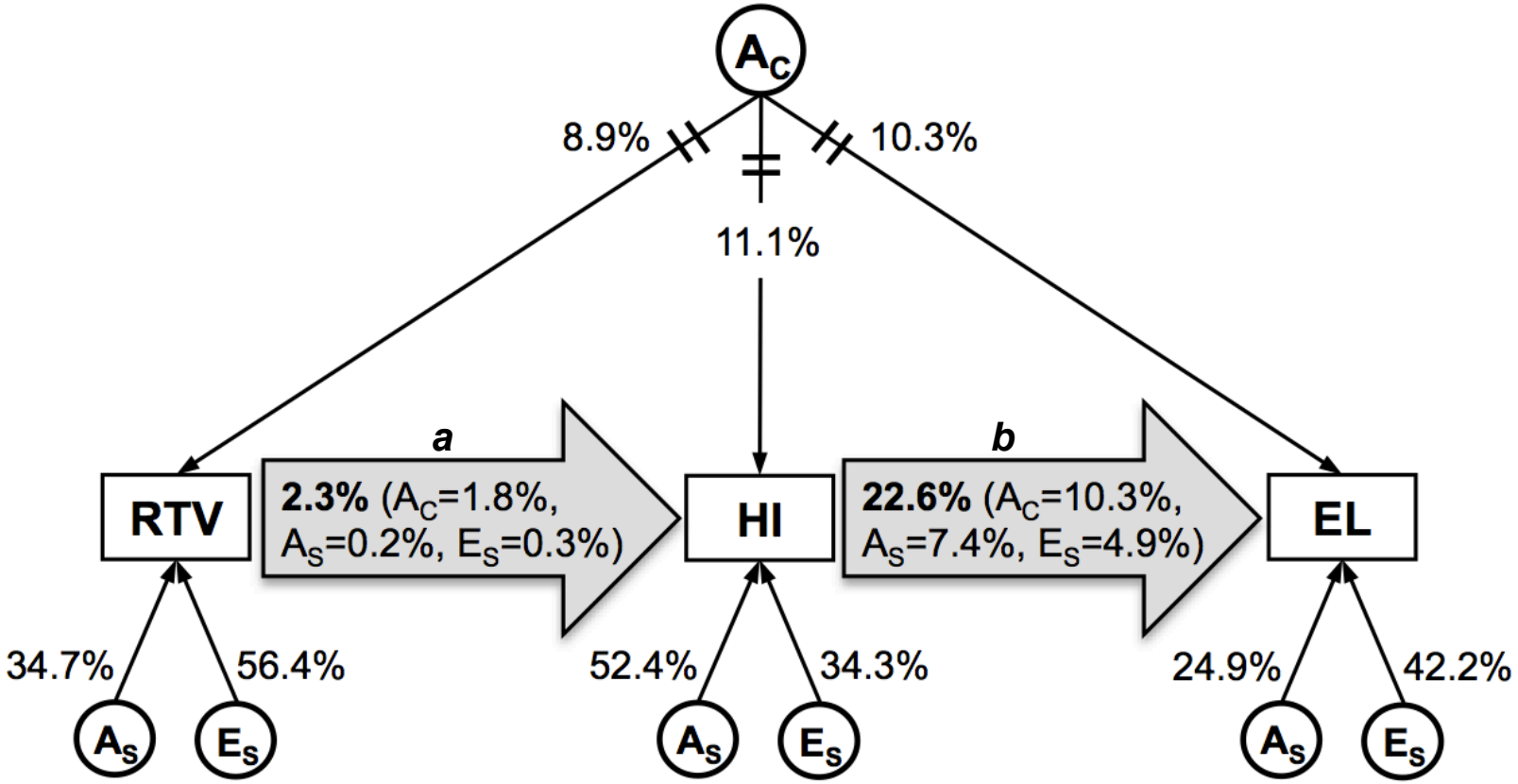
Note: Fit statistics for genetic models with either hyperactivity-impulsivity (Set 1) or inattention (Set 2) included as the mediator variable; -2LL = log likelihood statistic; df = degrees of freedom; AIC = Akaike's Information Criterion; BIC = Bayesian Information Criterion; χ^2 = likelihood ratio test (LRT) for difference between full and restricted models; Δdf = difference in degrees of freedom for LRT; *p* = significance of LRT; best fitting models denoted in **bold**.

Table 6.8. Residuals for the best-fitting genetic mediation models

Model	A_C	A_S	E_S	*a*	*b*	V
Genetic set 1						
RTV	0.96 (0.67, 1,19)	1.89 (1.58, 2.16)	2.41 (2.24, 2.61)	-	-	10.30
HI	0.96 (0.67, 1,19)	2.09 (2.34, 1.84)	1.69 (1.55, 1.85)	0.07 (0.01, 0.13)	-	8.33
EL	0.96 (0.67, 1,19)	1.49 (1.77, 1.17)	1.94 (1.77, 2.12)	-	0.39 (0.32, 0.46)	8.92
Genetic set 2						
RTV	1.07 (0.67, 1.19)	1.85 (1.54, 2.13)	2.41 (2.23, 2.60)	-	-	10.37
IA	1.07 (0.67, 1.19)	1.45 (1.04, 1.79)	2.00 (1.82, 2.20)	0.12 (0.06, 0.19)	-	7.24
EL	1.07 (0.67, 1.19)	1.83 (1.54, 2.09)	1.93 (1.76, 2.11)	-	0.17 (0.09, 0.25)	8.84

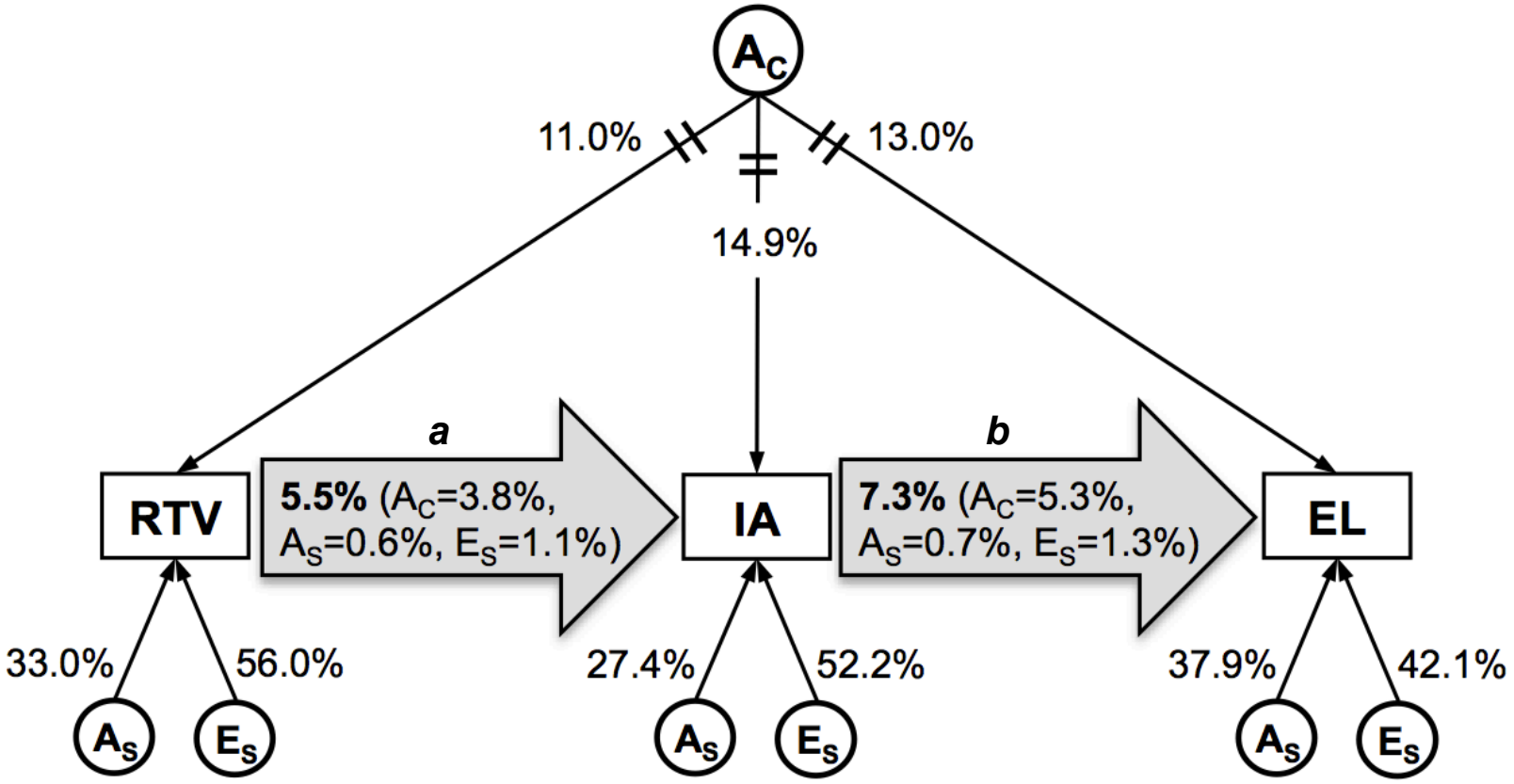
Note: Residual estimates for genetic and environmental parameters (A_C, A_S, E_S) and mediation paths (*a, b*) for each phenotype, with 95% confidence intervals in parentheses; V = estimates of phenotypic variance derived from expected covariance matrix in Mx; RTV = reaction time variability; HI = hyperactivity-impulsivity; IA = inattention; EL = emotional lability; 95% confidence intervals in parentheses; standardised estimates depicted in figures 6.5 and 6.6, with calculations presented in Appendix D.

Figure 6.5. Standardised parameter estimates for the best-fitting genetic mediation, including hyperactivity-impulsivity as the mediator



Legend: All factor loadings constrained to be equal for twins 1 and 2, so presented for one twin only; RTV = reaction time variability, HI = hyperactivity-impulsivity, EL = emotional lability; A_C = common genetic factor, constrained to explain an equal amount of the variance in RTV, HI and EL; A_S = specific genetic factor loading onto individual phenotypes; E_S = specific non-shared environmental factor loading onto each phenotype; grey arrows display the proportion of variance in HI explained by RTV (*a,* first arrow), and the proportion of variance in EL explained by HI (*b,* second arrow); bold text in arrow denotes total proportion of variance explained, values in parentheses indicate the amount of variance attributable to loadings of A_C, A_S, or E_S on the preceding variable.

Figure 6.6. Standardised parameter estimates for the best-fitting genetic mediation, including inattention as the mediator



Legend: All factor loadings constrained to be equal for twins 1 and 2, so presented for one twin only; RTV = reaction time variability, IA = inattention, EL = emotional lability; A_C = common genetic factor, constrained to explain an equal amount of the variance in RTV, IA and EL; A_S = specific genetic factor loading onto individual phenotypes; E_S = specific non-shared environmental factor loading onto each phenotype; grey arrows display the proportion of variance in IA explained by RTV (*a,* first arrow), and the proportion of variance in EL explained by IA (*b,* second arrow); bold text in arrow denotes total proportion of variance explained, values in parentheses indicate the amount of variance attributable to loadings of A_C, A_S, or E_S on the preceding variable.

6.4.6 Genetic mediation modelling

All model fit statistics are presented in Table 6.7. In the first set of genetic mediation models, hyperactivity-impulsivity was included as the mediator. To test the significance of mediation, paths *a* and *b* were dropped in turn (models 2 & 3) and simultaneously (model 4). Models 2-4 were a significantly worse fit than model 1, indicating that the mediation paths were important in explaining the covariance between RTV, hyperactivity-impulsivity and emotional lability. Next, to test the significance of the common genetic liability, A_C was dropped (model 5). This resulted in a significant deterioration in fit when compared to model 1, indicating that the common genetic liability also made an important contribution to phenotypic covariance. Finally, to test the significance of the common non-shared environmental liability, E_C was dropped (model 6). This resulted in no change in fit compared to model 1, indicating that common non-shared environmental influences did not contribute to covariation. The same pattern of findings emerged in the second set of models, where inattention was included as the mediator. The best fitting model in both instances was therefore model 6. This indicates that there was significant mediation of the association between RTV and emotional lability via the symptoms of ADHD (either HI or IA), in addition to a common genetic liability.

Residuals for the best-fitting models (Table 6.8) were used to calculate standardised estimates of the variance explained by the common versus mediated liability (Figures 6.5 and 6.6; for calculations see Appendix D). In the first model (Figure 6.5), RTV explained 2.3% of the total variance in hyperactivity-impulsivity; however this primarily reflected a mediated influence of the common genetic liability. Hyperactivity-impulsivity explained 22.6% of the total variance in emotional lability; of which 10.3% reflected effects of the common liability, while the remaining 12.3% reflected mediated effects of genetic and environmental influences specific to hyperactivity-impulsivity. This finding indicates that a common liability accounted for covariance between RTV, hyperactivity-impulsivity and emotional lability, while there was an additional, unique etiological association between hyperactivity-impulsivity and emotional lability. In the second model (Figure 2c), RTV explained 5.5% of the total variance in inattention, which primarily reflected a mediated influence of

the common genetic liability. RTV accounted for 7.3% of the total variance in emotional lability, which also primarily reflected mediated influence of the common genetic liability. This suggests that covariation between RTV, inattention and emotional lability was almost entirely accounted for by a common genetic liability as opposed to mediated effects.

6.5 DISCUSSION

Chapter 5 of this thesis presented evidence of a common aetiology, primarily genetic in origin, for the co-occurrence of ADHD and emotional lability symptoms among a population-based sample of child and adolescent twins. The research in this chapter sought to investigate the underlying mechanisms, testing whether the same cognitive performance deficits linked to ADHD also accounted for emotional lability via two sets of statistical analyses.

The first set of analyses demonstrated weak but significant associations of emotional lability with cognitive performance deficits, including slower mean reaction time (MRT), greater reaction time variability (RTV), commission errors (CE), and impaired digit span forwards (DSF) and backwards (DSB). These variables have been consistently linked to ADHD in phenotypic and familial/genetic analyses (Andreou et al., 2007, Frazier-Wood et al., 2012, Kuntsi et al., in 2013, Kuntsi et al., 2010, Kuntsi et al., 2009, Marco et al., 2009, Paloyelis et al., 2009, Rommelse et al., 2008, Uebel et al., 2010a). However, when controlling for hyperactivity-impulsivity and inattention, all associations were attenuated to a non-significant level. This directly replicates previous work conducted within IMAGE, a parallel clinical study of ADHD probands and their families (Banaschewski et al., 2012), indicating no direct association between emotional lability and the core cognitive deficits implicated in ADHD. This leads to the rejection of the hypotheses suggesting that emotional lability may arise as a direct result of deficits in state regulation, executive functioning or delay aversion (Barkley, 2010, Skirrow et al., 2009, Sonuga-Barke, 2005). Results are instead consistent with a mediation hypothesis, in which cognitive performance might impact on emotional lability via ADHD (Banaschewski et al., 2012).

The second set of analyses applied structural equation models as formal tests of mediation. Phenotypic mediation models confirmed the preliminary results, indicating no direct association between RTV and emotional lability when accounting for the symptoms of ADHD (either hyperactivity-impulsivity or inattention). Genetic mediation models indicated that these mediation paths accounted for specific associations between RTV and ADHD symptoms, and between ADHD symptoms and emotional lability. However, the covariance between RTV and emotional lability was not accounted for by mediated genetic/environmental effects and was instead due to a common genetic liability. This can be seen as representing pleiotropic genetic effects (Kendler and Neale, 2010, Kendler et al., 1993a), whereby the same sets of genes are associated with a range of cognitive and behavioural difficulties, including RTV, ADHD and emotional lability.

The lack of direct association between cognitive performance and emotional lability symptoms appears inconsistent with the results of treatment studies in adults with ADHD, in which hyperactive-impulsive, inattentive and emotional lability symptoms correlate in their response to medication (Marchant et al., 2011a, Marchant et al., 2011b, Reimherr et al., 2005a, Reimherr et al., 2005b, Reimherr et al., 2007, Rosler et al., 2010, Wender et al., 1985). The co-action of medication has led to the expectation that the same neurobiological substrates will underpin ADHD and emotional lability, although this was not supported at the cognitive level based on the results reported here, or in prior clinical research (Banaschewski et al., 2012, Surman et al., 2013). Medication does lead to improvements in cognitive performance in ADHD, although these are less consistent than the improvements in behavioural symptoms and are less prominent for executive than non-executive functions (Swanson et al., 2011).

One implication is that a common neurobiological basis linking ADHD and emotional lability might be reflected in cognitive functions other than those measured in this study. Further research is therefore required to characterise alternative cognitive processes that could account for the association between emotional lability and ADHD, such as emotion recognition and processing (Surman et al., 2013). Another, important consideration is whether cognitive performance deficits actually play a causal role in the development of ADHD

symptoms or ADHD as a disorder. The endophenotype hypothesis of ADHD is based on a causal assumption, although in practice causality is rarely tested (Kendler and Neale, 2010). As mentioned in this chapter, the alternative hypothesis to causality is one of pleiotropy, whereby the same liability influences a range of traits but without necessitating a causal link. In theory, pleiotropic effects could account for the entirety of the association between cognitive performance and ADHD; although the findings presented in this chapter indicate that both a common liability (pleiotropy) and mediation pathways (causality) were important in accounting for the association between RTV and ADHD symptoms. Nonetheless further research is required that directly addresses the causal association between cognitive performance and ADHD, including carefully controlled treatment studies and longitudinal research.

Throughout this study the two ADHD dimensions were analysed separately. Hyperactivity-impulsivity and inattention were strongly correlated, in line with prior estimates obtained from this sample (Kuntsi et al., in 2013, Wood et al., 2011b). Similarly, both ADHD dimensions were significantly correlated with RTV. Emotional lability was associated with both ADHD dimensions, but was significantly more strongly related to hyperactivity-impulsivity. This is the first study from SAIL to examine emotional lability, however this result is consistent with those obtained from different samples (see chapter 5).

Divergence of the association of emotional lability with the two ADHD dimensions was further reflected in the mediation modelling, including genetic models. These identified a unique genetic association between emotional lability and hyperactivity-impulsivity, but not between emotional lability and inattention. In contrast, inattention was more strongly genetically related to RTV, although not significantly so. This finding can be interpreted in the context of other recent results from SAIL indicating a stronger genetic association of RTV with inattention than with hyperactivity-impulsivity (Kuntsi et al., in 2013). Taken together, these results may point towards a separation of attention-related processes, including RTV, from externalised emotions and behaviours. This is somewhat consistent with Barkley's assertion that inattention reflects a self-regulatory deficit and that hyperactivity-impulsivity and emotional lability

reflect an inhibitory deficit (Barkley, 2010); although the association of a cognitive index of inhibition (CE) with ADHD and emotional lability in this study was particularly weak. This further highlights the need to identify neurocognitive factors that can adequately index the correlated liability for symptoms ADHD and emotional lability.

There are several limitations that should be considered when interpreting the results presented here. Foremost is the fact that this study used cross-sectional data, meaning that causality cannot be inferred from the mediation models. The mediation models were used to test specific hypotheses regarding the phenotypic and genetic associations between cognitive performance, ADHD and emotional lability in the absence of longitudinal data. Nonetheless longitudinal analyses would have strengthened the conclusions and should be included in future studies. Experimental studies will also provide a further alternative and powerful approach for testing mediation (Kendler and Neale, 2010).

Four further limitations are seen in relation to the genetic modelling in this study. First, the genetic mediation models parameterised only additive genetic and non-shared environmental variance components, despite evidence of significant non-additive genetic influences on inattention in the univariate modelling. This approach was taken to simplify the genetic mediation models and also because a sample of this size has low power to detect genuine non-additive genetic effects (Rietveld et al., 2003). The true extent of additive genetic influences on each variable are therefore somewhere within the 95% confidence intervals reported.

Second, and related to the above, Levene's test for equality of variance indicated significantly greater variances among DZ than MZ twins for symptoms of inattention, confirmed using a constrained version of the multivariate saturated model (see section 2.3.5). When interpreted in conjunction with the low DZ cross-twin within-trait correlations for inattention this is suggestive of a contrast effect. However, it was decided not to fit models including contrast effects for several reasons. One is that the small sample size for SAIL limits power to detect contrast effects, as well as limiting the power to detect non-additive genetic influences (Rietveld et al., 2003). Another is that the contrast

effect is assumed to be a form of rater bias specific to parental reports (Simonoff et al., 1998), whereas the present study used a composite of parent and teacher ratings to assess inattention. Yet another is that previous studies using the SAIL data have failed to identify significant MZ/DZ variance differences based on univariate saturated models (Cheung et al., under review) and the prior convention within this sample has therefore been not to fit contrast effect models. The decision not to test for contrast effects further, for example in the univariate genetic model, therefore seems to be appropriate. This decision is also in line with the primary aim of this chapter, which was to fit mediation models rather than to test for evidence of rater bias and/or contrast effects. Nonetheless the pattern of variance and correlation differences is of relevance when interpreted alongside the other results in this thesis and the topic is therefore picked up again in the general discussion in chapter 8. As a general recommendation for further research, it would be interesting to examine the full extent of rater contrast effects in SAIL in future.

Third, this study did not compare the genetic mediation model to other multivariate models that might have better accounted for the association between RTV, ADHD and emotional lability symptoms. This was again consistent with the aim of testing a specific hypothesis and is also consistent with approach taken in previous twin analyses that used the same multivariate model (Kendler et al., 1993a).

Fourth, the mediation models tested here are likely too simplistic, having included only three variables to simplify the modelling. A more realistic scenario is one in which there are multiple pathways from genes to cognitive performance, to ADHD symptoms, and to emotional lability, likely also linking to other behavioural traits.

Despite these limitations, the present study provided an informative test of the associations between cognitive performance, hyperactivity-impulsivity, inattention and emotional lability, using a population-based sample of child twin pairs. The use of a genetically-sensitive design directly addresses limitations identified in previous research (Banaschewski et al., 2012). The results indicated that ADHD and emotional lability symptoms primarily co-occurred as a

result of shared genetic influences, as opposed to a mediated influence of cognitive processes on emotional lability. These results suggest a common liability, but with potentially different neurobiological pathways from genes to ADHD versus emotional lability behaviours. Nonetheless, because of the common liability, and due to the association of emotional lability with impairment (Anastopoulos et al., 2011, Skirrow and Asherson, 2013), clinicians should remain particularly vigilant when diagnosing and treating ADHD, with a view to helping children and their families to identify and additionally manage the difficulties associated with emotional lability symptoms.

7. TESTING THE POLYGENIC THEORY OF ADHD

7.1 OVERVIEW

Due to the high heritability and the population-wide distribution of ADHD symptoms, it is assumed that ADHD operates under a model of polygenic inheritance; whereby many common alleles of small effect confer an additive risk for both the clinical disorder and for quantitative trait scores. Yet the polygenic basis of ADHD remains poorly characterised. The aim of chapter 7 was to test the polygenic theory of ADHD using a genetic profile score method. Participants were drawn from several different samples. To generate the profile score, genome-wide association analyses were performed in 8 ADHD samples from the Psychiatric Genomics Consortium (PGC). The profile score was then tested for association with ADHD affection status via logistic regression in the International Multi-centre ADHD Genetics project (IMAGE) sample; and for association with ADHD symptoms and related traits via linear regressions using the sample from the Twins Early Development Study (TEDS) and a subset of TEDS participants who participated in the Study of Activity and Impulsivity Levels in children (SAIL). Logistic regression identified a significant association between the profile score and ADHD affection status in IMAGE, indicating the presence of a significant polygenic signal for ADHD. There were also significant associations of the profile score with symptoms of hyperactivity-impulsivity rated using the Conners Parent Rating Scale-Revised and teacher ratings using the Strengths and Difficulties Questionnaire hyperactivity scale in TEDS; and with symptoms of emotional lability from the Long Version of Conners' Parent and Teacher Rating Scales in SAIL. These findings support the polygenic theory of ADHD and suggest that common variants associated with the clinical disorder are also associated with quantitative traits including hyperactivity-impulsivity and emotional lability.

7.2 INTRODUCTION

Twin studies consistently estimate high heritability for ADHD, in the order of 70-80% (Nikolas and Burt, 2010), with the same genetic liability thought to

influence clinical cases and the expression of symptoms throughout the general population (Chen et al., 2008, Larsson et al., 2012a, Levy et al., 1997). Yet molecular genetic studies of ADHD have generally failed to identify specific genetic variants that contribute to the genetic risk identified in twin studies. Candidate gene studies have identified only a handful of consistent results (Brookes et al., 2006, Gizer et al., 2009, Li et al., 2006), with a few associations approaching the genome-wide significance threshold of $p < 5*10^{-8}$ (Dudbridge and Gusnanto, 2008). Genome-wide association studies (GWAS) have similarly failed to identify associations that surpass this threshold, including the largest meta-analytic study to date (Neale et al., 2010b).

There are several possible explanations for this so-called *"missing heritability"* (Maher, 2008), including non-additive genetic effects (i.e. dominant or epistatic interactions between alleles) and within-sample heterogeneity in genetic studies (Manolio et al., 2009). However the accepted wisdom is that ADHD likely operates under a model of polygenic inheritance, with many genes of small effect conferring an additive risk that cannot be uncovered without a substantial increase in statistical power (Franke et al., 2009). It is therefore believed that current GWAS are underpowered to detect common variants of low penetrance assumed to be associated with ADHD, and that this will remain the case until sample sizes in the region of N = 20,000 are obtained (Neale et al., 2008). However, this does not preclude tests of polygenic inheritance using existing genome wide association data.

One example of a polygenic method is *genome-wide complex traits analysis* (GCTA), used to estimate the additive genetic heritability of a phenotype based on all genotyped SNPs (Yang et al., 2010). The GCTA method has estimated significant SNP-wide heritability (SNP-h^2) of 28% (standard error = 0.023) for ADHD affection status, indicating that additive genetic effects can be detected when taking a polygenic approach (Cross-Disorder Group of the Psychiatric Genomics Consortium, in press). However, a separate study applying the GCTA method within a general population sample failed to identify significant SNP-wide heritabilities for a range of quantitative trait measures of ADHD (Trzaskowski et al., in press, summarised in Table 7.1) These results are suggestive of differences in the polygenic influences on ADHD as a clinical

disorder and a quantitative trait; however these analyses do not test whether the same set of SNPs associated with ADHD affection status are also associated with ADHD symptoms.

Table 7.1 GCTA estimates for ADHD quantitative trait scores and ADHD affection status in the Twins Early Development Study (replicated from Trzaskowski et al., in press)

Trait	SNP-h^2	*SE*
CPRS-R - ADHD	0.00	0.12
CPRS-R - HI	0.06	0.12
CPRS-R - IA	0.00	0.12
SDQ - P	0.00	0.12
SDQ - T	0.05	0.15
SDQ - C	0.00	0.12

Note: CPRS-R = Conners Parent Rating Scale - Revised; ADHD = total ADHD symptom score; HI = hyperactivity-impulsivity symptom score; IA = inattention symptom score; SDQ = Strengths and Difficulties Questionnaire hyperactivity scale, completed by parents (P), teachers (T), or children (C); SNP-h^2 = estimate of SNP-wide heritability; SE = standard error; large standard errors relative to the estimates of SNP-h^2 indicate non-significance.

Another technique used to detect polygenic inheritance is the *profile score* method, as employed by the International Schizophrenia Consortium (Purcell et al., 2009). The polygenic basis of schizophrenia was tested by splitting the available data into discovery and target datasets. Genome-wide association analysis was then run in the discovery set and the results across single nucleotide polymorphisms (SNPs) summed to generate a score of reference ("risk") alleles. This score explained approximately 3% of the total variance in schizophrenia affection status in the target set, demonstrating a significant polygenic signal for schizophrenia from the measured SNP genotypes. The polygenic signal for schizophrenia was also predictive of bipolar disorder. The profile score method therefore allows common single nucleotide polymorphisms (SNPs) to be tested for association with a phenotype or across phenotypes *en-masse*, using the available data from existing GWAS. This makes it a complementary approach to GCTA.

To date, three profile score analyses have been conducted for ADHD. The first was a cross-disorder study from the Psychiatric Genomics Consortium (PGC), which found significant polygenic associations between autism, bipolar disorder, major depression and schizophrenia, but not ADHD (Cross-Disorder Group of

the Psychiatric Genomics Consortium, 2013). The second identified significant associations of profile scores for schizophrenia and bipolar disorder with ADHD, explaining up to 0.58% of the variance in ADHD affection status (Hamshere et al., 2013b). The third study generated a profile score using a large sample of children and adolescents with ADHD as the discovery set, using all SNPs associated at the threshold $p < 0.5$ (Hamshere et al., 2013a). This score was significantly associated with ADHD affection status in an independent training sample, but explained just 0.098% of the variance in affection status. The profile score was also significantly associated with conduct problems within the same sample.

Studies employing the profile score method have therefore identified a polygenic basis for ADHD as a clinical disorder and suggest that there may also be polygenic associations with other psychiatric comorbidities. However, there are several limitations associated with the existing profile score studies. First, the study by Hamshere et al. (2013) used a single threshold to select SNPs to use when generating a profile score for ADHD (i.e. all SNPs from the discovery sample at the threshold $p < 0.5$ were used to generate the profile score). It is therefore unclear whether the predictive power of the profile score can be improved by taking different thresholds based on more stringent or more relaxed *p* values. Second, the strength of the polygenic predictions across profile score studies has been very weak (i.e. half a percent or less of the variance in ADHD explained). Since these prior studies were published larger samples for ADHD genetics studies have become available, and it remains to be seen whether this will increase the effect sizes predicted by profile scores for ADHD. Third, although there is some evidence of cross-disorder effects, no studies have yet tested a profile score for ADHD affection status for association with ADHD symptoms or related traits within the general population. This would provide a direct test of the quantitative trait hypothesis of ADHD and would address a limitation associated with the existing GCTA studies of ADHD.

Accordingly, the aim of the present study was to addressing these limitations, conducting further tests of the polygenic basis of ADHD using the profile score method. A range of significance thresholds for the selection of SNPs used to generate profile scores. Profile scores were generated in a larger dataset than

used previously, evaluating whether an increase in the size of the discovery set would improve the predictive value of the profile score for ADHD in an independent dataset. The profile score was tested for association with ADHD-related traits among the general population, including ADHD symptom ratings from different informants, measures of cognitive performance known to be associated with ADHD, and symptoms of emotional lability. These tests evaluated whether common variants associated with ADHD affection status were also associated with ADHD continuous symptom scores and related traits, as suggested by the results of family and twin studies (see sections 1.4, 1.6 and 1.8).

A number of hypotheses were investigated. First, it was hypothesised that the profile score generated in the discovery set would positively predict ADHD affection status in an independent ADHD case-control target dataset (i.e. that a higher profile score would distinguish ADHD cases from controls). Second, it was hypothesised that a higher profile score predict higher levels of ADHD symptomatology among the population target set. The direction of association was expected to be the same across the different ratings of ADHD symptoms. Third, it was hypothesised that a higher profile score would predict greater cognitive deficits (increased reaction time variability, increased number of commission errors on an inhibitory control task, and lower IQ). The association of individual genetic markers with ADHD or the related traits is not reported in this chapter, since it is the focus of separate, ongoing analyses within the PGC and TEDS.

7.3 METHODS

7.3.1 Sample and measures

Samples were obtained from the Psychiatric Genomics Consortium (PGC) ADHD subgroup, the Twins Early Development Study (TEDS) and the Study of Activity and Impulsivity Levels in children (SAIL). All samples are described in detail in the methods chapter of this thesis (section 2.2). The PGC ADHD sample consisted of nine different sub-samples, eight of which were used to create a discovery set for the generation of a polygenic score. The remaining

PGC sample was the International Multi-centre ADHD Genetics project (IMAGE), which was used as the proband target set for testing the polygenic score for association with ADHD affection status. The TEDS sample was used as a second target set to test the polygenic score for association with continuous ADHD symptom scores and related traits in a general population sample. The SAIL sample is a subset of TEDS, for whom cognitive performance and emotional lability were assessed. The number of participants across samples is presented in Table 7.2. DNA collection and the processing of genomic data are described in the methods chapter (section 2.4).

Table 7.2 Number of participants across studies

	N
PGC discovery sets	
CHOP	358 probands from trios
PUWMA	702 probands from trios
IMAGE 2	892 cases, 7,086 controls
Canada	170 probands from trios
China	1,014 cases, 932 controls
Germany	495 cases, 1,298 controls
Spain	616 cases, 435 controls
ROI/UK	727 cases, 1,801 controls
Proband target set	
IMAGE	783 probands from trios
Population target set	
TEDS	3,152 individuals
SAIL	330 individuals

Note: CHOP = Children's Hospital of Philadelphia, a US-based study; PUWMA = Pfizer-funded study from the University of California, Los Angeles, Washington University, and Massachusetts General Hospital; IMAGE 2 = International Multi-centre ADHD Genetics project 2; ROI = Republic of Ireland; UK = United Kingdom; N gives number of cases and controls, or for data trios the number of probands; for TEDS (SAIL) the number of genotyped individuals is presented.

The phenotypes of interest varied across the datasets. Within the PGC discovery set and the IMAGE (the proband target set), the phenotype of interest was ADHD affection status, diagnosed using DSM-IV criteria following research diagnostic interviews by the groups contributing to the consortium PGC ADHD datasets (see section 2.2.5). Within the TEDS sample (the population target set)

ADHD symptom scores were examined based on different informant ratings. These included ratings of hyperactivity-impulsivity, inattention and total ADHD symptoms using the Conners' Parent Rating Scale – Revised (CPRS-R), and parent, teacher and child self-ratings of ADHD using the SDQ hyperactivity scale. A multi-rater composite was additionally examined, which took the mean of parent, teacher and self-ratings using the SDQ. This composite was only generated for cases where parent, teacher and self-ratings using the SDQ were available. Details on these measures are provided in section 2.2.1. A subset of TEDS participants were included in SAIL and assessed across cognitive performance tasks. The cognitive variables of interest were reaction time variability (RTV), commission errors (CE) and IQ, selected because they were shown to share significant genetic correlations with ADHD in family and twin model fitting analyses (Kuntsi et al., in 2013, Kuntsi et al., 2010, Wood et al., 2010a, Wood et al., 2011b). Emotional lability was also assessed in SAIL, based on a composite score derived from emotional lability items of the CPRS-R:L and CTRS-R:L. Details of the SAIL measures are provided in section 2.2.4.

7.3.2 Statistical analyses

The polygenic analyses used imputed genomic data from the PGC and TEDS, passed through the respective QC pipelines (see section 2.4.2). The PGC data were imputed using the 1000 Genomes Project reference set, providing information on over 40 million markers for 2,186 phased haplotypes from the full 1000 Genomes Project dataset (1000 Genomes Project, 2013). This large number of variants included SNPs and structural variants with minor allele frequencies of 1% or higher, derived from sequencing. The TEDS data were imputed using Central European HapMap phase 2 and 3 SNP data as a haploid reference panel, in addition to using Wellcome Trust Case/Control Consortium 2 (WTCC2) control SNP data as a diploid reference panel. These panels do not have the same high density SNP coverage as the sequenced data from the 1000 Genomes Project, meaning that the imputed TEDS data included less SNPs. The number of SNPs across samples is detailed in Table 7.3. Full details on the imputation procedures are provided in section 2.4.2. The imputation and pre-processing of data was not conducted as part of this thesis.

Table 7.3 Number of SNPs across studies

	Post-imputation	Prune SNP quality	Prune LD
PGC discovery sets			
CHOP	40,273,813	4,876,566	-
PUWMA	40,275,990	5,612,904	-
IMAGE 2	40,258,828	3,720,861	-
Canada	40,280,632	4,506,509	-
China	40,283,324	2,887,538	-
Germany	40,273,813	5,333,783	-
Spain	40,280,632	5,663,169	-
ROI/UK	40,273,813	4,597,346	-
Proband target set			
IMAGE	40,262,315	4,838,002	503,526
Population target set			
TEDS (including SAIL)	1,724,384	1,560,533	91,563

Note: The number of SNPs in each column is the number retained; the PGC samples, including IMAGE, were imputed using the 1000 Genomes reference set and thus included more SNPs than TEDS, which was imputed using the HapMap 3 reference set; the prune for SNP quality removed poorly imputed SNPs from the PGC datasets (*info* < *0.95*) and from TEDS removed all SNPs that failed tests of Hardy-Weinberg Equilibrium, SNP missingness and minor allele frequency (*HWE* < *0.000001, geno* > *0.05, maf* < *0.05*); the prune for linkage disequilibrium (LD) removed one SNP per pair when pairwise R^2 > 0.2; for efficiency, only the target sets were pruned for LD.

7.3.2.1 Generating the polygenic scores

The analyses described from here onwards were completed as part of this thesis. All genomic analyses were run across a Linux-based cluster computer at the MRC Social Genetic and Developmental Psychiatry Centre, King's College London, UK. This enabled computationally demanding analyses to be split into low intensity jobs and run simultaneously across multiple computer nodes. Generation of the polygenic score took place in several stages.

First, separate genome-wide association analyses (GWAS) were conducted for the eight PGC samples included in the discovery set. Analyses were implemented in Plink version 1.07 (Purcell, 2013, Purcell et al., 2007) using the command: *--dosage*. The dosage command read in dosage data and performed association analysis in a logistic regression framework, comparing expected allele frequencies for each SNP in cases and controls. For the five case/control samples (IMAGE 2, China, Germany, Spain, ROI/UK), analyses included twenty

principal components (PCs) as covariates to account for population stratification (generated by the PGC, as described in section 2.4.2). This large number of PCs ensured parity with other, ongoing PGC analyses of ADHD and additionally accounted for any stratification effects that could have occurred as a result of sex (Psychiatric GWAS Consortium ADHD subgroup, in preparation). For the remaining three samples (CHOP, PUWMA, Canada), case/pseudo-control data were derived from family trios (see section 2.4.2). Cases and pseudo-controls are perfectly matched with regard to genetic background, thus eliminating population stratification and rendering the inclusion of PC covariates superfluous for these samples (Benyamin et al., 2009).

Second, the results of individual GWAS were pruned for imputation quality. All output files from GWAS included an R^2 quality metric for each individual SNP, for which an R^2 value closer to 1.00 indicated better expected quality of imputation. Poorly imputed SNPs can potentially increase measurement error and reduce overall statistical power; therefore an imputation quality threshold of $R^2 > 0.95$ was employed. This stringent threshold has been used in previous polygenic analyses (Simonson et al., 2011). Pruning resulted in the loss of an average of 88.5% of the total number of imputed SNPs, as summarised in Table 7.2.

Third, a meta-analysis was performed to combine pruned results from the eight individual GWAS, using the Plink command: *--meta*. This command ran meta-analysis on all SNPs present across two or more samples. A model with fixed effects was fit to the data based on the assumption that heterogeneity across studies was controlled for by the inclusion of PC covariates. Nonetheless, to test for potential confounding factors arising from the inclusion of Han Chinese cases and controls alongside data from individuals of European ancestry, a second meta-analysis was run excluding the Chinese sample. The results of both meta-analyses were used to generate profile scores that could be compared when applied to target dataset. The number of SNPs retained after meta-analysis was 6,324,739 when including the Chinese data and 6,252,034 when excluding the Chinese data. The precise results of the individual GWAS and the two meta-analyses (e.g. top SNPs, regions of interest) are not reported here, since genome-wide mega analysis of ADHD is the focus of a separate,

ongoing project (Psychiatric GWAS Consortium ADHD subgroup, in preparation). Nonetheless, it should be noted that no single marker surpassed the genome-wide significance threshold of $p=5*10^{-8}$ (Dudbridge and Gusnanto, 2008).

Using results from the two genome-wide meta-analyses, profile scores were generated for the proband target set (IMAGE) and the population target set (TEDS/SAIL) using the Plink command: *--score* (see Box 2.1, section 2.4.1). Profile scores were generated at various thresholds based on the *p* value for association of each individual SNP with ADHD affection status in the discovery meta-analyses. Nine *p* value thresholds were imposed: $p = 1.00$, $p < 0.80$, $p < 0.50$, $p< 0.10$, $p < 0.05$, $p < 0.01$, $p < 0.001$, $p < 0.0001$, and $p < 0.00001$. These thresholds mirror those used in previous research (Evans et al., 2009). By comparing a range of significance thresholds it was possible to assess whether variants giving rise to small versus large significance values in GWAS contributed to the risk for ADHD. This provides some indication of the overall power of the discovery sets; as samples sizes of discovery sets increase the strongest signal will tend to be found within increasingly stringent significance thresholds.

Prior to generating the profile scores in IMAGE, data were pruned for imputation quality using the procedures described above for the PGC discovery set. The well-imputed SNP set was then pruned to remove SNPs in high linkage disequilibrium (LD). Plink does not allow data in dosage format to be pruned for LD; thus the 1000 Genomes Project reference panel was downloaded, transferred into Plink binary input files using the vcf to ped file converter available from the 1000 Genomes Project website (1000 Genomes Project, 2013), and pruned for LD in Plink using the command: *--indep-pairwise 100 5 0.2*. This command examined the pairwise association between SNPs within a sliding window shifted in stepwise fashion, removing one SNP per pair when the pairwise association violated a predefined R^2 threshold. The parameters included specified the window size (100 SNPs), how far to move the window in each step (5 SNPs), and the threshold at which to prune ($R^2 > 0.2$). The 1000 Genomes Project reference set included 40,318,245 markers, of which 17,442,603 were in high LD. Markers that were high in LD but featured in the

well imputed SNP list for IMAGE were excluded from analyses, leaving a total of 503,526 well-imputed SNPs in relative linkage equilibrium that were used to generate the profile score for IMAGE (see Table 7.3).

The TEDS data were similarly prepared by pruning for SNP quality and LD, albeit via different processes to those described for IMAGE. Because the TEDS data were in standard (non-dosage) format, SNP quality was pruned for in Plink by imposing thresholds for Hardy-Weinberg equilibrium (HWE < $1*10^{-6}$), genotype missingness (< 5% missing), minor allele frequency (MAF > 5%), and individual missingness (< 5% missing). An initial 1,724,384 were available, however the imposition of these thresholds led to the exclusion of 163,851 SNPs. No individuals were excluded based on missingness. TEDS data were then pruned for LD using the Plink command: *--indep-pairwise 100 5 0.2* (as described above). This led to the exclusion of a further 1,468,970 SNPs. The final dataset thus included 91,563 SNPs in relative linkage equilibrium used to generate the profile score (see Table 7.3).

7.3.2.2 Testing the polygenic scores

The profile scores were tested for association with ADHD affection status, ADHD symptom scores and associated traits across the proband and population target sets. All analyses were implemented as regression models using STATA version 10.1 (StataCorp., 2007). Prior to analyses, the polygenic scores were standardised to a mean of 0 and standard deviation of 1 to aid interpretation. Continuous phenotypes (e.g. ADHD symptom scores, cognitive performance) were similarly standardised, having been transformed to normality where required using the Stata command *lnskew0*. All regression models were followed by the command *vif* to estimate the variance inflation factor (VIF), an index of multi-collinearity. VIF < 2 indicated no problems of multi-collinearity for any of the combinations of variables included in analyses. These steps ensured that the basic statistical assumptions of linear regression were met (Acock, 2008). There were several stages to analyses.

First, the profile score at varying significance thresholds was tested for association with ADHD affection status in IMAGE (the proband target set),

testing whether reference alleles for common SNPs can be used to discriminate ADHD cases from controls. The different thresholds of profile score were included as predictor variables in logistic regression models with ADHD affection status (case/pseudo-control status) as the outcome variable. The statistics used to compare the various thresholds of profile score were the *z* score, *p* value and Nagelkerke's pseudo R^2, a measure of effect size that simulates R^2 from linear regression. Use of these statistics enabled direct comparison with a recently published polygenic analysis of ADHD (Hamshere et al., 2013a). The *p* values reported for the effects of the profile scores are one-tailed, in line with the unidirectional hypothesis that a greater number of reference alleles would increase the likelihood of ADHD. Scores calculated with and without the Chinese sample were compared, with only the best set of predictors taken forward for the remaining analyses to reduce the burden of multiple testing. Odds ratios and their 95% confidence intervals were additionally examined to compare relative strength of the profile scores generated with and without Chinese sample.

Second, the different thresholds of profile score were included as predictors of ADHD symptoms in TEDS (the population target set) in a series of hierarchical linear regressions. This tested the hypothesis that a greater number of score alleles were associated with levels of ADHD quantitative trait scores within the general population. These analyses additionally enabled comparison of different informant ratings of ADHD symptoms, building on the twin analyses conducted in chapter 3. The first step of hierarchical regressions entered the covariates age and sex, in addition to 8 principal components to control for genetic diversity within TEDS (see section 2.4.2). The second step entered the profile score, with each threshold of profile score modelled in turn. The statistics used to assess significance were the *t* score, *p* value and the standardised regression coefficient (beta, β). Again, these statistics were chosen for consistency with prior research (Hamshere et al., 2013a), with one-tailed *p* values presented in line with the unidirectional hypotheses.

Third, the different thresholds of profile score were included as predictors of cognitive performance in the SAIL subsample of TEDS, testing the hypothesis that a greater number of ADHD reference alleles would predict greater deficits

in cognitive performance. The profile score was also used to predict emotional lability symptoms in SAIL. This provided a molecular genetic replication of the quantitative genetic analyses reported in Chapter 6, testing the hypothesis that a greater number of ADHD score alleles would predict greater levels of emotional lability. Hierarchical linear regressions were implemented as described above.

7.4 RESULTS

7.4.1 Prediction of ADHD affection status in IMAGE

7.4.1.1 Descriptive statistics in IMAGE

Descriptive statistics for the proband target set (IMAGE) are presented in Table 7.4. The mean scores for ADHD symptoms are in line with those reported in previous analyses using the same sample (Banaschewski et al., 2012, Sobanski et al., 2010). The probands were aged 5-18 years and were predominantly male (88%). This sex ratio is typical of clinical samples of children and adolescents with ADHD in the UK (Hamshere et al., 2013a).

Table 7.4 Descriptive statistics for the IMAGE sample

	M	SD	N	%
ADHD	73.80	7.70	783	-
HI	74.83	8.38	783	-
IA	69.25	7.47	783	-
Age (years)	10.74	2.74	783	-
Male	-	-	689	88.0
Female			94	12.0

Note: descriptive statistics reported in order to characterise the IMAGE sample; ADHD = composite ratings of total ADHD symptoms made using the Conners Parent Rating Scale - Revised (CPRS-R) and Conners Teacher Rating Scale - Revised CTRS-R; HI = composite ratings of hyperactivity-impulsivity; IA = composite ratings of inattention; all scales had been transformed into *t* scores to provide standardised estimates.

7.4.1.2 Distribution of the profile score

The different thresholds of polygenic score were approximately normally distributed, both when excluding and including the Chinese data (in Stata: skewness = 0±1, kurtosis = 3±1). Boxplots of the different thresholds of score are presented for the cases and pseudo controls in Figure 7.1 (excluding Chinese data) and Figure 7.2 (including Chinese data). In both figures the scores appear higher among ADHD cases than among the pseudo controls, although this difference is slightly more prominent when including Chinese data. Both figures indicate some outliers (denoted by dots); however these were retained in analyses since there was no reason to assume that they represented invalid data points (Acock, 2008).

Figure 7.1 Box plots for the different thresholds of profile score as predictors of affection status in IMAGE - excluding Chinese data

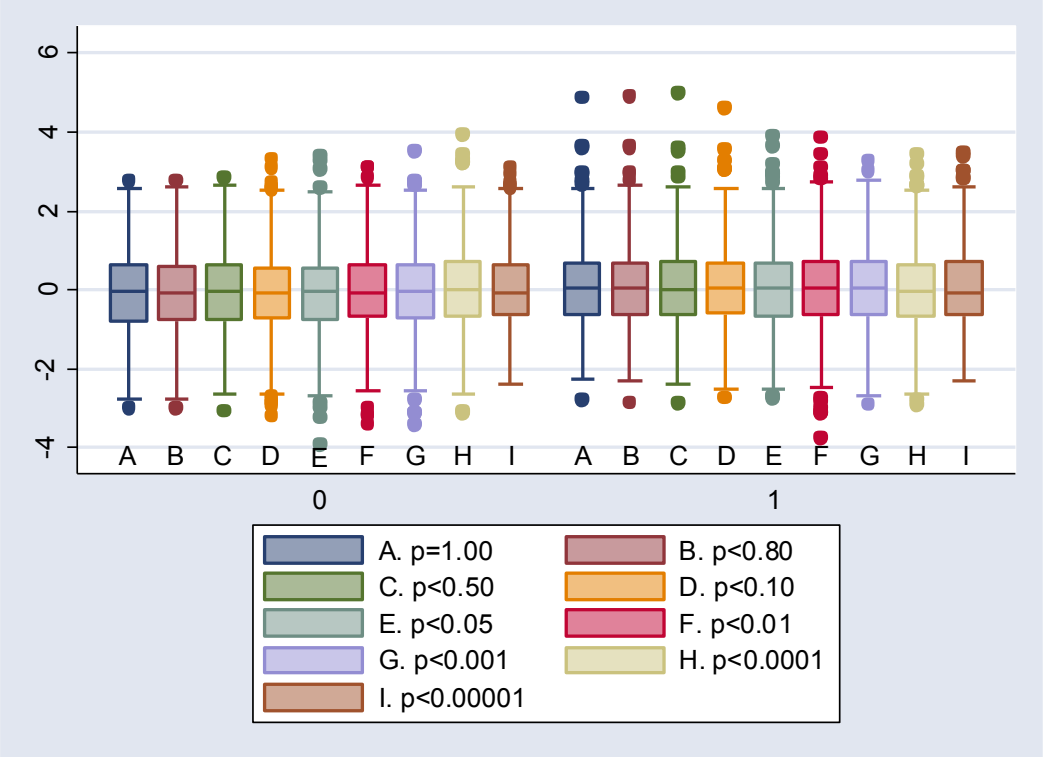
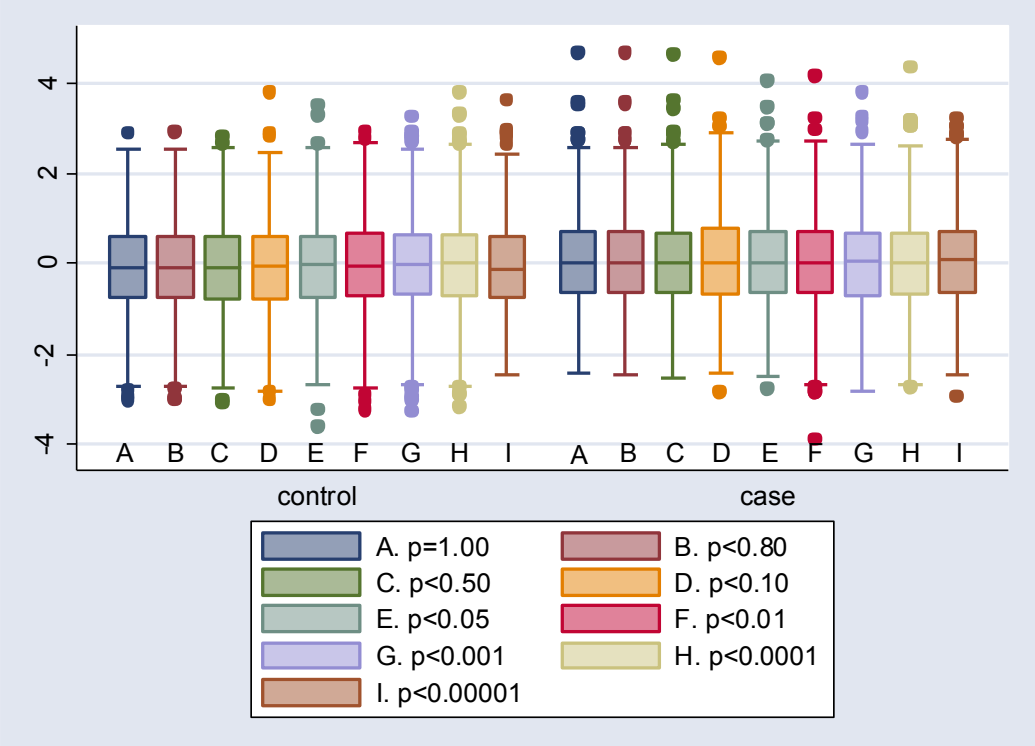


Figure 7.2 Box plots for the different thresholds of profile score as predictors of affection status in IMAGE - including Chinese data



Legend Figures 7.1 & 7.2: Labels A-I indicate the threshold of profile score; boxes represent the interquartile range of the data; subdividing lines inside boxes indicate median scores; whiskers extend to data points 1.5 times the interquartile range of the upper and lower quartiles; dots denote outliers.

Table 7.5 Logistic regressions predicting ADHD affection status in IMAGE

Threshold	Exclude Chinese data (N= 14,580)				Include Chinese data (N= 16,526)			
	R^2	*z*	*p*	OR (CI)	R^2	*z*	*p*	OR (CI)
p=1.00	0.004847	2.41	.008	1.13 (1.02, 1.25)	0.005705	2.62	.005	1.14 (1.03, 1.26)
p<0.80	0.004657	2.36	.009	1.13 (1.02, 1.24)	0.005484	2.56	.005	1.14 (1.03, 1.26)
p<0.50	0.004483	2.32	.011	1.12 (1.02, 1.24)	0.005120	2.48	.007	1.13 (1.03, 1.25)
p<0.10	0.003767	2.12	.017	1.11 (1.01, 1.23)	0.004121	2.21	.014	1.12 (1.01, 1.24)
p<0.05	0.003021	1.89	.030	1.10 (1.00, 1.22)	0.002155	1.60	.055	1.08 (0.98, 1.20)
p<0.01	0.002878	1.84	.033	1.10 (0.99, 1.21)	0.001621	1.38	.084	1.07 (0.97, 1.18)
p<0.001	0.002596	1.74	.041	1.09 (0.99, 1.21)	0.000958	1.06	.145	1.06 (0.96, 1.17)
p<0.0001	0.000052	-0.25	.598	0.99 (0.89, 1.09)	0.000415	0.70	.243	1.04 (0.94, 1.14)
p<0.00001	0.000000	-0.01	.504	1.00 (0.91, 1.10)	0.003859	2.12	.017	1.11 (1.01, 1.23)

Note: N = number of subjects used to generate profile score; R^2 = Nagelkerke's pseudo R^2; *z* = *z* test statistic; *p* = 1-tailed significance; OR = odds ratio for prediction of affection status, with 95% confidence intervals in parentheses; all statistics derived from logistic regressions with robust standard errors.

7.4.1.3 Logistic regressions

Logistic regressions compared the different thresholds of profile score for their ability to predict ADHD affection status. The regression models did not include age or sex covariates, since cases and pseudo-controls are by definition perfectly matched for these variables. The results are presented in Table 7.5 for the logistic regression models with robust standard errors to account for potential outlier effects. The models without robust standard errors (not reported) gave a virtually identical set of results.

Using the discovery set without Chinese data, the best predictor was the profile score at the threshold p = 1.00, which explained 0.48% of the variance in affection status (Nagelkerke's pseudo R^2 = 0.004847). The predictive value of the profile score declined at increasingly stringent thresholds, becoming non-significant at the threshold $p < 0.05$. This suggests that nominally associated reference alleles from GWAS conferred an additive risk for ADHD beyond that accounted for by the top GWAS hits.

The discovery set including the Chinese data similarly indicated that the best predictor was the profile score at the threshold p = 1.00, but with a slightly stronger effect size than was found in analyses excluding Chinese data (0.57% of the variance explained, Nagelkerke's pseudo R^2 = 0.005705). This likely reflects increased power afforded by an increase in sample size of N = 1,946 and suggests that the strength of the polygenic signal for ADHD should improve further as larger samples become available in future. The profile score similarly became non-significant at the threshold $p < 0.05$ but improved at the final threshold of $p < 0.00001$. These results suggest that nominally associated reference alleles continued to predict ADHD affections status, but that the most significant hits from GWAS made an additional contribution to ADHD affection status. The significance of the score in the top banding could further reflect increased statistical power when including the Chinese data in the discovery set GWAS.

Overall, the R^2 values were larger for six out of nine thresholds of profile score when including the Chinese data; however overlapping confidence intervals for

the odds ratios indicate that these were not significant differences. Nonetheless, on the basis of these results only the profile scores generated with the Chinese data were taken forward for inclusion in the population-based analyses.

7.4.2 Prediction of quantitative trait scores in TEDS

7.4.2.1 Descriptive statistics in TEDS

Descriptive statistics for the population target set (TEDS/ SAIL) are presented in Table 7.6. Mean scores for the parent-rated Conners scales and the parent, teacher and self-rated SDQ are reported for the TEDS sample based on data collected at ages 10-12 years (mean = 11.36). These are similar to mean scores reported previously for the entire TEDS twin sample (Greven et al., 2011c; see also chapter 3). As expected for a population-based sample, the sex ratio was close to 50:50. Measures of emotional lability and cognitive performance (RTV, CE, IQ) are reported for the subset of TEDS who participated in SAIL. Data were collected when children were aged 7-10 years (mean = 8.84). Mean scores for cognitive performance and emotional lability are similar to those reported previously for the entire SAIL twin sample (see chapter 6).

7.4.2.2 Phenotypic correlations

Pairwise correlations between the continuous measures of ADHD symptoms and cognitive performance in TEDS and SAIL are presented in Table 7.7. Symptoms of hyperactivity-impulsivity and inattention were moderately correlated based on the CPRS-R (r = 0.53), as were the different informant ratings of ADHD symptoms using the SDQ (r = 0.30 to 0.47). These findings are consistent with prior research (Greven et al., 2011c; see also chapter3). The cognitive performance variables correlated modestly with one another and with ADHD. In particular, the correlations of RTV with hyperactive-impulsive and inattentive ADHD symptoms were somewhat weaker than those reported in chapter 6. This likely reflects the fact that the cognitive and behavioural data used here were collected at different time points. The correlations of emotional lability symptoms with ADHD symptoms followed a similar pattern.

Table 7.6 Descriptive statistics for TEDS/ SAIL

	M	SD	N	%
TEDS				
ADHD	9.54	8.36	2693	-
HI	4.10	4.26	2692	-
IA	5.44	5.94	2695	-
SDQ - P	2.78	2.26	2694	-
SDQ - T	2.07	2.41	2138	-
SDQ - C	3.48	2.27	2691	-
SDQ - M	2.76	1.77	1952	-
Age (years)	11.36	0.67	2874	-
Male - TEDS	-	-	1313	45.7
Female - TEDS	-	-	1561	54.3
SAIL				
RTV	625.13	338.52	315	-
CE	103.11	34.26	320	-
IQ	108.84	15.50	324	-
EL	2.86	2.67	287	-
Age (years) - SAIL	8.84	0.68	324	-
Male - SAIL	-	-	148	45.7
Female - SAIL	-	-	176	54.3

Note: descriptive statistics reported for raw data; ADHD = total ADHD symptom score from the CPRS-R; HI = hyperactivity-impulsivity symptom score; IA = inattention symptom score; SDQ = Strengths and Difficulties Questionnaire hyperactivity scale, completed by parents (P), teachers (T), or self-rated by children (C); M denotes SDQ composite derived by taking the mean of the parent, teacher and self-rated SDQ scores; RTV = reaction time variability; CE = commission errors; IQ = WISC-III score; EL = composite measure of emotional lability derived from CPRS-R and the Conners Teacher Rating scale CTRS-R.

Table 7.7 Pairwise phenotypic correlations for continuous variables in the population target set (TEDS and SAIL)

	ADHD	HI	IA	SDQ - P	SDQ - T	SDQ - C	SDQ - M	RTV	CE	IQ
HI	0.83									
	(<.001)									
IA	0.90	0.53								
	(<.001)	(<.001)								
SDQ - P	0.73	0.61	0.68							
	(<.001)	(<.001)	(<.001)							
SDQ - T	0.35	0.26	0.34	0.34						
	(<.001)	(<.001)	(<.001)	(<.001)						
SDQ - C	0.44	0.37	0.40	0.47	0.30					
	(<.001)	(<.001)	(<.001)	(<.001)	(<.001)					
SDQ - M	0.66	0.54	0.61	0.78	0.68	0.79				
	(<.001)	(<.001)	(<.001)	(<.001)	(<.001)	(<.001)				
RTV	0.14	0.07	0.16	0.12	0.10	0.10	0.21			
	(.015)	(.209)	(.005)	(.042)	(.117)	(.078)	(.001)			
CE	0.16	0.15	0.13	0.14	0.18	0.13	0.20	0.14		
	(.006)	(.012)	(.022)	(.015)	(.004)	(.021)	(.001)	(.011)		
IQ	-0.16	-0.16	-0.13	-0.25	-0.21	-0.10	-0.24	-0.04	-0.06	
	(.006)	(.005)	(.023)	(<.001)	(.004)	(.087)	(.001)	(.457)	(.312)	
EL	0.33	0.36	0.23	0.25	0.12	0.10	0.20	0.18	0.18	-0.08
	(<.001)	(<.001)	(.002)	(<.001)	(.071)	(.099)	(.002)	(.003)	(.003)	(.205)

Note: ADHD = total ADHD symptoms from the Conners' Parent Rating Scale - Revised (CPRS-R); HI = hyperactivity-impulsivity ratings from the CPRS-R; IA = inattention ratings from the CPRS; SDQ = Strength and Difficulties Questionnaire hyperactivity scale, completed by parents (P), teachers (T) or children (C), or a mean composite of parent, teacher and child ratings (M); RTV = reaction time variability; CE = commission errors; EL = emotional lability; all correlations run using transformed/standardised data; table provides Pearson correlation coefficient (*r*) with two-tailed *p* value in parentheses.

7.4.2.3 Distribution of the profile score

The different thresholds of profile score appeared normally distributed within the population sample target set (in Stata: skewness = 0±1, kurtosis = 3±1), although examination of box plots revealed a number of outliers (Figure 7.3). For most thresholds of the profile score the spread of outliers was approximately symmetrical (i.e. a similar amount at each tail of the distribution). However at the threshold $p<0.00001$ all outliers were high profile scores. An inspection of the data revealed that a number of individuals (n = 694) did not carry any reference alleles at this threshold and thus had a profile score of zero, throwing the high profile scores into sharp relief. Due to the presence of outliers all hierarchical linear regressions used robust standard error estimates.

7.4.2.4 Covariate effects

Prior to conducting regression analyses the different thresholds of profile score were tested for association with the covariates age and sex, and the eight principal components. There were no associations with age or sex ($p > 0.05$), but there were significant associations with some principal components (see Appendix E). This indicates that the principal components controlled for some stratification effects. All covariates were retained in subsequent analyses.

7.4.2.5 Prediction of ADHD symptom scores

The different thresholds of profile score were used to predict symptoms of total ADHD, hyperactivity-impulsivity and inattention in the population target set (TEDS). The first step in hierarchical regressions revealed significant associations of the covariates age, sex and study site with each of the dependent variables (see Appendix E). The second step entered each threshold of profile score in turn. Results are presented in Table 7.8. There were no significant associations of the profile score with total ADHD symptoms or with inattention. However, the profile score at the thresholds $p = 1.00$ and $p < 0.80$ was significantly associated with symptoms of hyperactivity-impulsivity ($\beta = 0.038134$ and $\beta = 0.037060$). This indicates that the same set of reference alleles associated with ADHD affection status in the PGC discovery set,

predicted greater levels of hyperactive-impulsive ADHD symptoms within the population target set.

7.4.2.6 Prediction of different informant ratings of ADHD symptoms

The different thresholds of profile score were then used to predict different informant ratings of ADHD symptoms. After controlling for covariates (Appendix E), the profile score was not significantly associated with parent or child ratings of ADHD made using the SDQ (Table 7.9). However, there was a significant association with teacher ratings at the threshold $p < 0.10$ ($\beta = 0.037804$). The profile score was not significantly associated with composite SDQ ratings (Table 7.10).

7.4.2.7 Prediction of cognitive performance

The different thresholds of profile score were next used to predict cognitive performance in a subset of the population target set (SAIL). After controlling for covariates (Appendix E), the profile score was not significantly associated with RTV, CE or IQ (Table 7.11).

7.4.4.6 Prediction of emotional lability

The final set of regressions examined the association of the profile score symptoms of emotional lability (also in SAIL). After controlling for covariates (see Appendix E) the profile score at the thresholds $p < 0.0001$ and $p < 0.00001$ was significantly associated with emotional lability ($\beta = 0.136564$ and $\beta = 0.101860$), see Table 7.12). This indicates that some of the more strongly associated score alleles for ADHD affection status in the PGC discovery set also predicted greater levels of emotional lability among the population target set.

Table 7.8 Linear regressions predicting ADHD symptom scores in the population target set (TEDS)

Threshold	Total ADHD symptoms			Hyperactive-impulsive symptoms			Inattentive symptoms		
	β	*t*	*p*	β	*t*	*p*	β	*t*	*p*
p=1.00	0.019498	1.05	0.148	0.038134	2.03	0.022	0.003200	0.17	0.432
p<0.80	0.018944	1.02	0.155	0.037060	1.97	0.025	0.003030	0.16	0.436
p<0.50	0.012084	0.65	0.260	0.030442	1.62	0.053	-0.003520	-0.19	0.574
p<0.10	0.012045	0.65	0.258	0.025360	1.34	0.090	-0.001081	-0.06	0.523
p<0.05	0.000533	0.03	0.489	0.011266	0.59	0.277	-0.008614	-0.45	0.675
p<0.01	0.002483	0.13	0.448	0.011056	0.58	0.281	-0.000891	-0.05	0.519
p<0.001	0.002733	0.15	0.442	0.008829	0.47	0.319	0.001719	0.09	0.463
p<0.0001	-0.016497	-0.88	0.812	-0.006973	-0.37	0.644	-0.027670	-1.52	0.936
p<0.00001	-0.014213	-0.79	0.784	0.008811	0.48	0.317	-0.031325	-1.71	0.956

Note: ADHD symptom scores derived from the Conners' Parent Rating Scale - Revised (CPRS-R); all analyses control for age, sex and principal components; β = beta coefficient from regression, *t* = *t* test statistic; *p* = one-tailed significance; regressions estimated robust standard errors.

Table 7.9 Linear regressions predicting different informant ratings of ADHD in the population target set (TEDS)

Threshold	Parent SDQ			Teacher SDQ			Child SDQ		
	β	*t*	*p*	β	*t*	*p*	β	*t*	*p*
p=1.00	0.012380	0.66	0.255	0.024785	1.18	0.120	-0.004099	-0.22	0.587
p<0.80	0.012828	0.68	0.248	0.025694	1.22	0.112	-0.004564	-0.24	0.596
p<0.50	0.011029	0.58	0.281	0.027069	1.29	0.098	-0.005593	-0.30	0.617
p<0.10	0.008687	0.47	0.319	0.037804	1.81	0.036	0.010587	0.57	0.285
p<0.05	-0.002064	-0.11	0.544	0.031992	1.52	0.064	0.001761	0.09	0.463
p<0.01	-0.009946	-0.54	0.704	0.034782	1.63	0.052	-0.020221	-1.07	0.858
p<0.001	-0.006884	-0.37	0.644	-0.016448	-0.78	0.781	-0.035613	-1.86	0.969
p<0.0001	-0.009599	-0.51	0.697	-0.013914	-0.66	0.745	-0.061881	-3.19	1.000
p<0.00001	-0.006813	-0.37	0.644	0.015930	0.78	0.781	-0.024719	-1.33	0.908

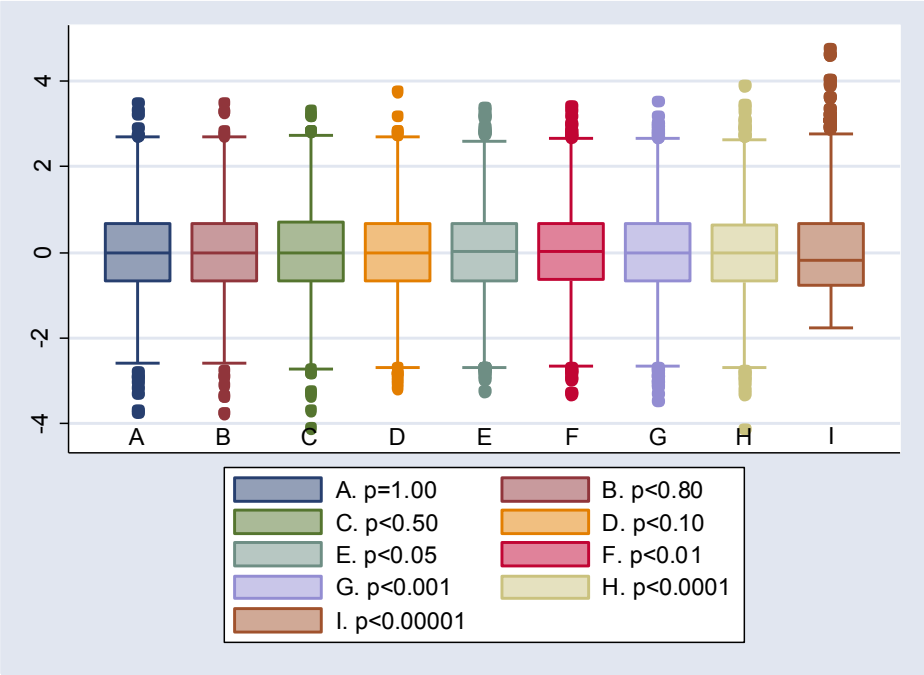
Note: ADHD symptom ratings derived from the SDQ hyperactivity scale, completed by parents, teachers and self-rated by children; all analyses control for age, sex and principal components; *β* = beta coefficient from regression, t = t test statistic; *p* = one-tailed significance; regressions estimated robust standard errors.

Table 7.10 Linear regressions predicting the SDQ multi-informant composite in the population target set (TEDS)

	β	*t*	*p*
p=1.00	0.030427	1.37	0.086
p<0.80	0.031199	1.40	0.081
p<0.50	0.029013	1.31	0.095
p<0.10	0.028918	1.37	0.086
p<0.05	0.024698	1.14	0.108
p<0.01	0.003119	0.15	0.442
p<0.001	-0.016402	-0.77	0.780
p<0.0001	-0.040993	-1.91	0.972
p<0.00001	-0.013026	-0.62	0.731

Note: β = beta coefficient from regression, *t* = *t* test statistic; *p* = one-tailed significance; regressions estimated robust standard errors.

Figure 7.3 Box plots for the different thresholds of profile score in TEDS / SAIL



Legend Labels A-I indicate the threshold of profile score; boxes represent the interquartile range of the data; subdividing lines inside boxes indicate median scores; whiskers extend to data points 1.5 times the interquartile range of the upper and lower quartiles; dots denote outliers.

Table 7.11 Linear regressions predicting cognitive performance in the population target set (SAIL)

Threshold	RTV			CE			IQ		
	β	*t*	*p*	β	*t*	*p*	β	*t*	*p*
p=1.00	-0.006125	-0.12	0.546	0.013722	0.22	0.413	0.023099	0.40	0.655
p<0.80	-0.006159	-0.12	0.546	0.012036	0.19	0.423	0.024244	0.42	0.663
p<0.50	-0.033557	-0.65	0.541	-0.002344	-0.04	0.515	0.052908	0.89	0.814
p<0.10	-0.022034	-0.41	0.658	0.040348	0.69	0.247	0.027300	0.42	0.620
p<0.05	-0.045071	-0.82	0.793	-0.017841	-0.31	0.621	0.016905	0.27	0.606
p<0.01	0.048466	0.85	0.197	-0.019995	-0.37	0.643	0.034727	0.54	0.707
p<0.001	-0.055492	-1.03	0.847	-0.097267	-1.77	0.961	0.077813	1.38	0.916
p<0.0001	-0.004678	-0.08	0.534	0.000445	0.01	0.497	0.059707	1.04	0.850
p<0.00001	0.010516	0.19	0.427	0.078800	1.45	0.075	0.017619	0.31	0.623

Note: RTV = reaction time variability; CE = commission errors; IQ assessed using the WISC-III; details on all cognitive measures are available in section 2.2.4; all analyses control for age, sex and principal components; β = beta coefficient from regression, *t* = *t* test statistic; *p* = one-tailed significance; regressions estimated robust standard errors.

Table 7.12 Linear regressions predicting emotional lability in the population target set (SAIL)

	β	*t*	*p*
p=1.00	0.026565	0.45	0.326
p<0.80	0.025599	0.44	0.332
p<0.50	0.017092	0.30	0.383
p<0.10	0.007305	0.14	0.446
p<0.05	-0.019628	-0.36	0.644
p<0.01	0.070460	1.22	0.113
p<0.001	0.011773	0.21	0.417
p<0.0001	0.136564	2.37	0.010
p<0.00001	0.101860	1.69	0.046

Note: β = beta coefficient from regression, *t* = *t* test statistic; *p* = one-tailed significance; regressions estimated robust standard errors.

7.5 DISCUSSION

This chapter examined the polygenic basis of ADHD. A profile score comprising multiple reference ("risk") alleles associated with ADHD was generated in a large discovery set of ADHD cases and controls. Results indicated a significant association of the profile score with ADHD affection status in an independent proband target set (IMAGE) and significant associations with symptoms of ADHD and emotional lability in a second, general population target set (TEDS/SAIL).

The polygenic association with ADHD affection status is in line with another recent study that used a partially overlapping sample (Hamshere et al., 2013a). In that study, the IMAGE, IMAGE 2, PUWMA and CHOP samples were combined to form the discovery set, based on the meta-analysis of ADHD GWAS (Neale et al., 2010b). A score was generated using all SNPs associated with ADHD in the discovery set at a threshold of $p < 0.5$, and explained 0.098% of the variance in ADHD affection status in an independent target set comprising some of the PGC sample from ROI/UK.

The previous study was the first to formally demonstrate a polygenic signal for ADHD, however the present set study builds on those results threefold. First, the present set of analyses compared a range of thresholds of profile score to

determine whether reference alleles that were strongly or weakly associated with ADHD in GWAS contributed to the risk for ADHD affection status in an independent sample. The best threshold was p = 1.00, indicating that a signal comprising all reference alleles associated with ADHD in the discovery set resulted in the best prediction of ADHD affection status in the independent target set. At more stringent thresholds, the association between the profile score and ADHD affection status was attenuated, eventually becoming non-significant. This is consistent with the pattern of results reported for other complex phenotypes (Evans et al., 2009, Purcell et al., 2009) and suggests that alleles of very small effect, which were not significantly associated with ADHD in GWAS at the stringent threshold $p<5*10^{-8}$ may have conferred an increased risk for the disorder in the IMAGE sample. However, when using the discovery set that included Chinese data, the most stringent threshold of profile score (p < 0.00001) was also significantly associated with affection status. This suggests that there may be an additional effect of some of the more strongly associated alleles from GWAS in predicting ADHD.

Second, the present study included a larger sample than the previous polygenic study, with 9,165 additional participants in the discovery set when excluding the Chinese sample and 11,111 additional participants when including the Chinese sample. Consistent with the assumption that larger sample sizes will increase power and thus the association of a profile score with ADHD, the best threshold of profile score (p = 1.00) explained 0.48% of the variance in ADHD affection status when excluding Chinese data and 0.57% when including Chinese data. This indicates that the best profile score generated in the present study explained more than five times the variance in ADHD affection status than did the score generated in the previous study (Hamshere et al., 2013a). The difference between the results of this study and those reported by Hamshere et al. (2013a) could reflect other methodological differences. For example, data were imputed differently in the study by Hamshere et al. when compared to the present study. Hamshere et al. also used a different threshold (p < 0.50) to select SNPs when generating a profile score, as discussed above. Nonetheless, the same threshold in this study explained 0.45% when excluding the Chinese data and 0.51% when including the Chinese data, indicating that the present analyses did identify a stronger polygenic effect.

Third, the present study tested the profile score for association with ADHD symptoms and related traits among a general population sample. These analyses indicated significant associations with parent ratings of hyperactivity-impulsivity from the Conners' Parent Rating Scale - Revised (CPRS-R) and with teacher ratings from the Strengths and Difficulties Questionnaire (SDQ) hyperactivity scale. This indicates that a score comprising reference alleles associated with ADHD affection status was also associated with ADHD symptoms in subjects from the general population, supporting prior research in suggesting the same underlying liability for ADHD as a disorder and as a continuous trait (Chen et al., 2008, Larsson et al., 2012a, Levy et al., 1997).

Fourth, the profile score was additionally associated with symptoms of emotional lability in the SAIL subset of TEDS, suggesting that the alleles associated with ADHD affection status also predicted greater levels of emotional lability. This finding might be related to the polygenic association between ADHD and conduct disorder reported by Hamshere et al (2012), since emotional lability is associated with a higher risk of oppositional behaviour and substance abuse disorders (Sobanski et al., 2010) which are both strongly associated with conduct disorder. This finding also provides a molecular genetic replication of the twin results reported in chapters 5 and 6, which showed a genetic association between ADHD and emotional lability.

In spite of these findings, the majority of associations reported in this chapter were non-significant. In particular, the profile score was only significantly associated with two measures of ADHD symptoms in the population target set (TEDS): hyperactivity-impulsivity assessed using the CPRS-R and teacher ratings using the SDQ. The association with all other measures of ADHD symptoms was non-significant. A highly similar pattern of results was also found in a polygenic study using the GCTA method in the same sample (Trzaskowski et al., in press; see Table 7.1). In that study, SNP-wide heritability estimates were 6% for symptoms of hyperactivity-impulsivity and 5% for teacher ratings using the SDQ, but 0% for all other measures of ADHD. This suggests that a greater amount of the variance in CPRS-R hyperactivity-impulsivity and the teacher SDQ could be attributable to polygenic influences. However it is crucial to note that all SNP-wide heritability estimates for ADHD-related behaviours in

the previous study were non-significant (as indicated by their large standard errors).

The non-significant results in the previous TEDS study are important as they suggest that additive genetic influences, when measured at the molecular level, did not account for any of the variance in ADHD symptom scores. The conclusion drawn in that study was that genetic influence across a range of behavioural traits could be non-additive in origin (Trzaskowski et al., in press). This conclusion is consistent with the results of some twin research, which suggests that certain ADHD rating scales are more likely to be influenced by non-additive genetic effects, including ratings of inattention and parent ratings of ADHD (Nikolas and Burt, 2010). However, it is difficult to align this theory with the results of polygenic analyses in clinical samples, which have demonstrated significant polygenic influences on ADHD, autism, bipolar disorder, major depression and schizophrenia when using the GCTA (Cross-Disorder Group of the Psychiatric Genomics Consortium, in press). One potential explanation is that there is a qualitative difference between ADHD as a disorder and ADHD trait scores among the population. An alternative explanation is that the behaviours measured using rating scales are under a greater level of non-additive genetic influence than are symptoms assessed via clinical interviews.

The present study failed to identify significant associations of the profile score with measures of cognitive performance among the population target set (SAIL). One potential explanation is low power, since the target set included a relatively small number of individuals. Yet this was not a problem when predicting symptoms of emotional lability. This difference could perhaps reflect the particularly strong phenotypic and genetic correlation between ADHD and emotional lability symptoms compared to the cognitive performance measures, such as RTV, as demonstrated in chapter 6 of this thesis. Another potential explanation is heterogeneity. Research suggests that there are individual differences in levels of RTV and CE among ADHD probands, leading to speculation that there are multiple cognitive pathways to ADHD (Halperin et al., 2008, Johnson et al., 2009, Nigg et al., 2005). Thus, if ADHD probands in the discovery set showed substantial heterogeneity in their profiles of cognitive performance then the power to detect genetic associations in the training set

may have been attenuated. The lack of polygenic association between ADHD symptoms and cognitive performance is consistent with exploratory findings from the IMAGE sample, which showed that a polygenic score generated within IMAGE was unable to predict scores for RTV, CE or IQ (Mould, unpublished data). However the previous analyses were severely limited by sample size, having generated a profile score data from around 500 individuals.

Had a significant association with the cognitive performance variables been observed in this study it would have provided some support for the endophenotype hypothesis of ADHD, which specifies that cognitive performance should be associated with the same genes that confer risk for ADHD (Kendler and Neale, 2010). The lack of association does not refute the endophenotype hypothesis but does indicate that further research is required. One strategy is to re-examine the association of the profile score with cognitive performance in a larger test sample, and/or to generate an improved discovery profile score using a larger discovery dataset. A second strategy is to generate a profile score for the cognitive performance variables and to use it as a predictor of ADHD. The first method should be possible in the near future since plans are underway to conduct a GWAS of cognitive performance using pooled international data (Asherson, 2013) and since larger GWAS datasets for ADHD are also being accrued. The second approach would likely require larger samples than currently exist; although this should be possible for IQ, which has been assessed across a number of large-scale studies. If significant association between a profile score and a measure of cognitive performance is observed, then a mediation model could be tested to determine whether cognitive performance deficits truly lie on the pathway between genes and behaviour (Kendler and Neale, 2010). Unfortunately, the non-significant associations in this chapter did not allow this final test of mediation versus pleiotropic effects.

A number of limitations exist that should be considered when interpreting these results and perhaps the most apparent of these is the issue of multiple testing. In this chapter, ADHD affection status in IMAGE and 11 different phenotypes in TEDS were tested for association with 9 different thresholds of profile score, substantially increasing the likelihood of a type I error. A legitimate concern, therefore, is that the reported associations may simply be due to chance.

Multiple testing was considered an acceptable limitation given the exploratory nature of the research in this chapter; however it is recommended that the issue be addressed fully in future research. One approach is to conduct tests of permutation to derive empirical significance levels that can account for multiple testing. Another approach is to conduct follow-up and replication studies.

There are also a number of other limitations related to the methods used to generate and test the polygenic signal. First, the inclusion of a Han Chinese sample alongside those of European ancestry is a limitation since it increased genetic heterogeneity within the discovery set. While this had the potential to cause false positive results due to population stratification effects, the problem of stratification was counterbalanced by the inclusion of a large number of principal components as covariates in analyses. Furthermore, the analyses herein compared the predictive value of profile scores generated when including and excluding the Chinese sample from the discovery set. Although pragmatic, this comparative approach revealed that inclusion of the Chinese sample improved the overall strength of the polygenic score when predicting ADHD. Nonetheless, future analyses should explore the impact of using data from accrued from different geographical locations. One approach would be to employ a cross-validation procedure, such that the profile score is developed and tested across different subpopulations to determine the effects of stratification on polygenic predictions.

Second, the use of family-based samples in the PGC discovery set and the proband target set (IMAGE) could be considered a limitation. Family-based samples are those derived from trios, where the within-family transmission of alleles from parents to offspring is examined. In the present set of analyses, the ADHD probands from trios were compared pseudo-controls derived from untransmitted parental alleles, enabling family-based data to be analysed in a similar manner to the population-based data (Cordell and Clayton, 2002, Cordell, 2004, Cordell et al., 2004). A strength of this approach is robustness against population stratification, in addition to other artifacts associated with the use of population-based samples (Cross-Disorder Group of the Psychiatric Genomics Consortium, in press). However, a concession is that family-based studies are less powerful precisely because the same marker is used to test

association while controlling for stratification (Benyamin et al., 2009). Another limitation is that family-based designs are more sensitive to genotyping errors (Benyamin et al., 2009). Specifically, family-based association studies show a systematic bias in the transmission of major alleles, likely due to errors in the process of calling minor alleles (Neale et al., 2008).

The limitations associated with the use of family-based data could have affected the generation of profile scores in the discovery set, where three samples included case/pseudo-controls from trios (CHOP, PUWMA, Canada). They could similarly have affected the testing of the profile scores in case/pseudo-controls from IMAGE. Consistent with this, the recent GCTA analysis of PGC data estimated lower SNP-wide heritabilities for ADHD among family-based studies using case/pseudo controls than among population-based studies (Cross-Disorder Group of the Psychiatric Genomics Consortium, in press). A more powerful approach in future might therefore be to test a profile score for its association with ADHD in a well-matched sample of cases and controls, genotyped at the same time to reduce artifacts. Yet despite these issues many authors continue to advocate the use of family-based analyses, particularly in the replication stages of analyses when the robustness against population stratification increases the likelihood of detecting genuine genetic effects (Benyamin et al., 2009, Cross-Disorder Group of the Psychiatric Genomics Consortium, in press). Moreover, analysis indicates that the imputation process is sufficient to remove allele-calling bias within the IMAGE sample (Mould, unpublished data), and that genotyping errors associated with imputation can be overcome via the imposition of stringent QC (Cross-Disorder Group of the Psychiatric Genomics Consortium, in press). The rigorous QC applied by the PGC during preparation of the data used herein (see section 2.4), coupled with the use of imputed data and stringent post-imputation pruning, should therefore have safeguarded against these limitations.

The analyses reported within this chapter are therefore subject to several limitations and are perhaps best thought of as preliminary. However in this sense they pave the way for future research, with important theoretical implications. First, the results support the polygenic theory of ADHD, indicating that common genetic variants do confer risk for the disorder and for symptoms

of ADHD among the general population. However, increasingly large samples will likely be required to detect larger polygenic effects in future, suggesting that the pooling and generation of additional data will facilitate research.

Second, analyses indicated that more lenient thresholds of profile score were better predictors of ADHD. Although these thresholds will contain a lot of noise (i.e. alleles unassociated with ADHD), the present results suggest that they also include alleles associated with the disorder. The implication is that far larger samples sizes are needed to allow true significant findings to reach genome wide levels of significance. Another way to capitalise on these findings is to conduct hypothesis-driven follow-up studies, for example enrichment studies examining gene systems in relation to ADHD. This approach was conducted for ADHD with some success in the detection of neurite outgrowth genes associated with ADHD (Poelmans et al., 2011). Follow-up analyses using the PGC, IMAGE and TEDS samples are now underway in a parallel project focusing on candidate gene systems (Roth-Mota, 2013).

Third, the results of this study, when interpreted alongside those of a recent GCTA study in TEDS, suggest that there may be non-additive genetic influences on ADHD symptoms that cannot be detected using simple association methods that are powered mainly for additive genetic effects. Accordingly, a follow-up to the analyses reported in this chapter is also underway, in which a machine learning approach is being used. Machine learning enables non-additive effects such as gene-gene interaction to be examined more readily and therefore has the potential to explain a greater proportion of the variance in ADHD affection status and ADHD symptoms scores if there may be non-additive genetic effects.

It would be foolhardy to claim that these results have direct translational clinical value at this stage: clearly the profile score generated here explained only a tiny fraction of the variance in ADHD affection status (little over half a percent), meaning that profile scores are currently of limited utility in terms of the clinical identification of ADHD cases. Despite the negative findings for the neurocognitive phenotypes, in the long run the greatest value from genetic findings is likely to be the way this methodology can be used to delineate the

processes by which genetic influences are translated into clinical phenotypes by identifying the neurobiological processes involved; and to find new ways to improve the function of the dysregulated systems.

8. GENERAL DISCUSSION

8.1 OVERVIEW

This chapter summarises the main findings of the empirical research conducted throughout this thesis. After summarising key findings and general limitations, the wider themes to emerge and their implications are explored. Potential implications for clinical practice and future directions are then considered.

8.2 AIMS AND KEY FINDINGS

8.2.1 Aim 1: Understand rater effects in twin studies of ADHD

The first aim of this thesis was to understand why different informant ratings of ADHD symptoms yield distinctive estimates of genetic and environmental effects in twin research. This was addressed in chapter 3 by examining the heritability of parent, teacher and self-ratings of ADHD and by comparing the extent to which common genetic and environmental influences contributed to different informant ratings.

There were two main findings. First, heritability estimates differed across informants, with lower estimates for child self-ratings (48%) than for parent (82%) or teacher (60%) ratings. Follow-up analyses indicated that the heritability of teacher ratings also differed depending on whether the same teacher or different teachers rated the behaviour of each twin from a pair (76% versus 49%). Second, multivariate modelling indicated shared and unique aetiological influences for the different informant ratings, suggesting shared but also rater-specific views of ADHD-related behaviours. Shared aetiological influences were represented by a common latent factor and were primarily genetic in origin, suggesting that the common aspect of different informant ratings indexes a highly heritable component of ADHD.

8.2.2 Aim 2: Explore the phenotypic and aetiological associations between ADHD and temperament

The second aim of this thesis was to explore the phenotypic and aetiological associations between ADHD symptoms and Cloninger's dimensions of temperament. This aim was addressed in chapter 4 via multivariate twin models examining the relationship of ADHD symptoms of hyperactivity-impulsivity and inattention with the temperament dimensions novelty seeking, harm avoidance, reward dependence and persistence, collected via self-report questionnaires during early adulthood.

There were two main findings. First, both the hyperactive-impulsive and inattentive dimensions of ADHD were significantly associated with the temperament dimension of novelty seeking at the phenotypic and genetic levels, suggesting that novelty seeking might be associated with a combined-type profile of ADHD. Second, the hyperactive-impulsive and inattentive dimensions were found to differ in their associations with harm avoidance and persistence. Harm avoidance was uniquely correlated with inattention but not hyperactivity-impulsivity at the phenotypic, genetic and environmental levels. Persistence was phenotypically correlated with both ADHD dimensions but with opposite directions of association; a positive association with hyperactivity-impulsivity was driven primarily by overlapping non-shared environmental influences, while a negative association with inattention was primarily due to overlapping genetic influences. This suggests that temperament might be used to characterise distinct ADHD profiles in future research.

8.2.3 Aim 3: Examine the relationship between symptoms of ADHD and emotional lability

The third aim of this thesis was to examine the association between the symptom dimensions of hyperactivity-impulsivity, inattention and emotional lability. This aim was addressed in chapters 5 and 6.

In chapter 5, multivariate modelling examined phenotypic and aetiological associations between the three symptom dimensions in child and adolescent

twin pairs aged 5-18 years. The results indicated significant phenotypic correlations between all three dimensions, but with a significantly stronger pairwise association of emotional lability with hyperactivity-impulsivity than with inattention. Genetic analyses indicated a shared aetiology for all three symptom dimensions, represented by a highly heritable common latent factor. This indicates that a substantial proportion of the genetic influences across phenotypes were shared, suggesting that emotional lability can perhaps be viewed as an integral component of a broader ADHD phenotype.

In chapter 6, analyses were extended to include measures of cognitive performance, testing whether common neurocognitive factors contributed to the association between ADHD and emotional lability. There were two main sets of results. The first set indicated weak but significant phenotypic associations between emotional lability and a number of cognitive performance deficits, including slower mean reaction time (MRT), greater reaction time variability (RTV), commission errors (CE), and impaired digit span forwards (DSF) and backwards (DSB). However, these associations were attenuated to a non-significant level when controlling for symptoms of ADHD, suggesting that ADHD mediated the association of emotional lability with cognitive performance. The second set of results focus on the association between RTV, emotional lability and ADHD symptoms of hyperactivity-impulsivity versus inattention. Phenotypic structural equation models confirmed that the symptoms of ADHD (either hyperactivity-impulsivity or inattention) completely mediated the association between RTV and emotional lability. Genetic mediation models indicated that these mediation paths accounted for specific associations between RTV and ADHD symptoms, and between ADHD symptoms and emotional lability. However, covariance between RTV and emotional lability was not accounted for by mediated aetiological effects, and was instead due to a common genetic liability. These results suggest that the association between ADHD and emotional lability is not due to shared cognitive deficits, and that the association of emotional lability with RTV is likely due to genetic pleiotropy.

8.2.4. Aim 4: Test the polygenic theory of ADHD

The fourth aim of this thesis was to test the polygenic theory of ADHD. This aim was addressed in chapter 7 by generating a polygenic profile score for ADHD affection status in a large discovery set of ADHD cases and controls. The profile score was then tested for association with ADHD affection status and with ADHD symptoms and related traits in two independent target sets.

There were four main findings. First, the profile score was significantly associated with ADHD affection status in an independent target set of ADHD probands. Second, the profile score for ADHD was significantly associated with ADHD symptoms among an independent target set from the general population; specifically, the profile score was associated with parent ratings of hyperactivity-impulsivity from the Conners' Parent Rating Scale - Revised (CPRS-R) and with teacher ratings from the Strengths and Difficulties Questionnaire hyperactivity scale (SDQ). These results suggest overlapping sets of genetic factors are associated with ADHD as a clinical disorder and as a quantitative trait. Third, the profile score for ADHD was significantly associated with symptoms of emotional lability in the population target set, suggesting a common molecular genetic basis for ADHD and emotional lability. The fourth main finding concerns a number of non-significant associations, which are of interest in helping to select phenotypes for inclusion in future genetic research.

8.3 GENERAL LIMITATIONS

8.3.1 Phenotyping

One limitation across studies is the potential bias associated with the definition, measurement and derivation of phenotypes (Farmer et al., 2002). Acceptable psychometric properties have previously been reported for the range of behavioural measures used in this thesis, including those used to assess ADHD (Chen and Taylor, 2006, Conners et al., 1998b, Conners et al., 1998a, DuPaul, 1981, Goodman, 2001, Larsson et al., 2011, Thapar et al., 2000), temperament (Brandstrom et al., 1998, Cloninger et al., 1993, Heath et al., 1994) and emotional lability (Parker et al., 1996, Westerlund et al., 2009). Moreover, the

different measures demonstrated generally acceptable levels of internal consistency when assessed in this thesis.

Nonetheless, the use of postal rating scales to collect behavioural data may have introduced bias by reducing the reliability of measures; for example, it is impossible to know for certain whether instructions were followed when questionnaires were completed (e.g. rating ADHD symptoms based on the past two weeks), or which informant was responsible for completing the measure. This is particularly relevant in light of the results in chapter 3, which highlight the potential limitations associated with the use of different informant ratings in twin research. One solution to overcome this in future is to use structured or semi-structured interviews to collect phenotypic data, as used in the IMAGE study and across other clinical samples from the PGC (chapter 7). However, the use of interview schedules is both costly and time-consuming. The psychometric properties of the cognitive performance measures used in this thesis have previously been found to be acceptable, with the strongest reliability found for composite measures of cognitive performance (Kuntsi et al., 2005a, Kuntsi et al., 2006). Further, due to the systematic methods of cognitive data collection they should be more objective than behavioural scales; therefore, a systematic method of behavioural data collection may facilitate future research.

Another potential limitation relates to item overlap and factor structures in multivariate research. The ADHD phenotype is relatively well defined, with strong support for a bi-factor structure that includes hyperactive-impulsive, inattentive and general symptom clusters (Martel et al., 2011, Martel et al., 2010c, Toplak et al., 2009, Toplak et al., 2012). However, the extent to which ADHD symptoms can be delineated from other traits remains unclear. This is particularly relevant to the research in chapter four, since item overlap could have accounted for the phenotypic and genetic associations of ADHD with temperament, in particular novelty seeking which includes items pertaining to impulsivity. Future research should seek to overcome this limitation by conducting exploratory factor analysis to demonstrate a separation of ADHD symptoms from Cloninger's dimensions of temperament prior to twin modelling. The issue of item overlap is less of a concern in chapters 5 and 6, since prior research has demonstrated a separation of hyperactive-impulsive, inattentive

and emotional lability symptoms into three dimensions (Parker et al., 1996, Westerlund et al., 2009) including research conducted in the sample used in chapter 5 (Chen, unpublished data). Yet the extent of item overlap between ADHD and emotional lability with oppositional defiant disorder and/or bipolar disorder symptoms was not established in this thesis. This will be an important step for future research because a common feature across all of these dimensions is impulsivity and irritable, volatile mood.

8.3.2 Sample representativeness

The twin research in chapters 3 to 6 used population-based samples, meaning that the results may not generalise to clinical cohorts. However, prior research has identified the same genetic liability for ADHD as a clinical disorder and as continuous trait (Chen et al., 2008, Larsson et al., 2012a, Levy et al., 1997), suggesting that the aetiological results in chapters 3 to 6 should extend to clinical samples. Still, this does not circumvent other limitations associated with the use of twin samples, such as differences from singletons in terms of physical characteristics (e.g. weight, height) and perinatal complications (Rijsdijk and Sham, 2002). Prior studies examining the generalisability of twin research to singletons have reported mixed results, with some studies finding differences between twins and singletons with regard to ADHD symptomatology (Levy et al., 1996) and others not (Johnson et al., 2002).

Future research can address this limitation in two ways. First, future twin studies could use bivariate Defries and Fulker (DF) extremes analysis (DeFries and Fulker, 1985, DeFries and Fulker, 1988) to examine the aetiological associations between ADHD and co-occurring traits among individuals with extreme symptom scores, who meet or are likely to meet ADHD diagnostic criteria. This would help to determine whether the aetiological overlap of ADHD with other traits, such as temperament and emotional lability, is the same in extreme groups when compared to the remainder of the population. Second, replication studies should be undertaken using non-twin and clinical samples. The latter of these approaches has already been employed to study associations between emotional lability and cognitive performance deficits in an

ADHD proband and sibling sample, with similar results to those reported in chapter 6 (Banaschewski et al., 2012).

A related issue is that the use of population-based samples resulted in skewed data for the behavioural measures of ADHD and emotional lability, and for many of the cognitive variables. Non-normal distribution of the data violates assumptions of the twin method and of the structural equation modelling package used to conduct analyses (Neale et al., 2006). The issue of non-normality was overcome in this thesis by transforming data, but could be addressed in future by using alternative measures of ADHD that yield a near-normal distribution among the general population, such as the Strengths and Weaknesses of ADHD and Normal Behavior Rating Scales (SWAN, Swanson et al., 2006).

It is also important to consider the representativeness of the clinical samples in polygenic analyses. Clinical samples were screened thoroughly to ensure that all cases met ADHD diagnostic criteria, but with different diagnostic assessments used across studies. This could have introduced heterogeneity, although it could be argued that the use of different diagnostic assessments is an accurate reflection of clinical practice, thus increasing external validity. It is also important to consider whether the use of pseudo-controls in genetic analyses reduced overall representativeness, particularly in the IMAGE sample used to test the profile score for association with ADHD affection status. The case/pseudo-control design is a family-based method and is generally considered more robust than population-based association studies (Benyamin et al., 2009); however future research could compare the predictive value of ADHD profile scores in family-based and population-based samples to determine whether the same results are found.

8.3.3 Use of cross-sectional data

The analyses reported in this thesis examined ADHD symptoms at a variety of ages from childhood through to early adulthood. However, all analyses were cross-sectional and did not make use of longitudinal data. This is a limitation for several reasons. First, the estimates of genetic and environmental effects in

twin research are made for a given population at a given point in time (Plomin et al., 2008). Replication is therefore required, not only in different populations but at different developmental stages so as to understand the aetiological relationship between ADHD and co-occurring traits across the lifespan. Second, the use of cross-sectional data means it was not possible to assess stability and change in these associations. Third, the use of cross-sectional data constitutes a specific limitation with regard to the results from chapter 6. Cross-sectional data limits the extent to which causality can be inferred from the mediation models. Future replications using longitudinal data are therefore particularly important as follow-up analyses for the mediation research, although even the use of longitudinal data is not sufficient to justify causal claims.

The use of cross-sectional data will not have impacted the molecular genetic results, since genes do not change over time. It would nonetheless be interesting to study developmental changes in gene regulation and expression or protein expression in ADHD in future, although this is a separate research question that would require access to genomic data from ribonucleic acid (RNA) rather than from DNA, or DNA taken at multiple time-points to capture epigenetic changes over time. The major limitation of such potential work is the restricted access to brain tissue, meaning that meaningful results can only emerge for gene/protein expression or epigenetic changes that are reflected in accessible peripheral tissues.

8.3.4 Additional limitations of the twin method

Although many of the issues associated with the twin method have already been discussed, some additional limitations remain. First, the twin studies in chapters 3 to 6 assume equal environments for MZ and DZ twins without having tested the equal environments assumption (EEA). Previous research suggests that the EEA is generally valid (see section 2.3), although it would be beneficial if future research tested the EEA across the different twin registers included in this thesis. Second, effects of chorionicity, gene-environment interactions, gene-environment correlations and assortative mating were not examined in this thesis, but could have potentially biased the estimates of genetic and environmental effects. However, because these factors tend to push parameter

estimates in different directions (i.e. some inflate estimates of genetic effects, others inflate estimates of environmental effects) any associated bias is likely to be minimal (Rijsdijk and Sham, 2002). These limitations further highlight the need to replicate the findings using non-twin samples. One novel way to achieve this in future studies is the use of Genome-wide Complex Traits Analysis (GCTA, Yang et al., 2010), which can provide estimates of univariate heritabilities and bivariate genetic correlations using measured genotypes in sufficiently large singleton samples of the different but related phenotypes included in this thesis.

8.4 THEMES AND IMPLICATIONS

8.4.1 Rater differences in heritability estimates

Rater differences in the heritability estimates for ADHD was a main finding from the research in chapter 3; parent ratings yielded heritability estimates of 82%, teacher ratings yielded estimates of 60%, and self-ratings yielded estimates of 48%. The high heritability for parent-rated ADHD symptoms was confirmed in chapter 5, with estimates of 83% for symptoms of hyperactivity-impulsivity and 77% for symptoms of inattention. These estimates are in line with those from prior twin research (Nikolas and Burt, 2010). The heritability of parent-rated emotional lability in chapter 5 was similar, estimated at 71%. Taken together, these findings indicate that parent ratings of ADHD-related behaviours in child and adolescent twins consistently yield high estimates of heritability.

Conversely, the lower heritability for self-rated ADHD symptoms in chapter 3 was confirmed in chapter 4, with heritabilities of 38% for hyperactivity-impulsivity and 40% for inattention among adult twin pairs. These heritability estimates are consistent with the results from other twin studies using self-ratings in adolescence and adulthood (Boomsma et al., 2010, Chang et al., 2013, Ehringer et al., 2006b, Haberstick et al., 2008, Kan et al., 2013, Larsson et al., 2012b, Martin et al., 2002, Schultz et al., 2006, Van Den Berg et al., 2006, Young et al., 2009b, Young et al., 2000), suggesting that the lower heritability of self-rated ADHD is a robust result. One previous interpretation was that ADHD symptoms in adults, which are primarily assessed via self

report, are less heritable than the symptoms in childhood (Boomsma et al., 2010). However, this conclusion seems implausible when the lower heritability of self-rated ADHD in early adolescence is considered, as reported in chapter 3. The fact that the genetic correlation between hyperactive-impulsive and inattentive symptoms appears stable across development (Greven et al., 2011c, Larsson et al., 2012b, McLoughlin et al., 2007) also casts doubt on this conclusion.

The alternative conclusion drawn in chapter 3 was that the use of two different informants (i.e. self-ratings) places a ceiling limit on estimates of heritability by reducing inter-rater agreement and overall reliability. This supposition garners support from the additional finding in chapter 3 that the heritability of teacher-rated ADHD was significantly lower when two different teachers rated the behaviours of each twin from a pair than when a single teacher rated the behaviour of both twins (49% versus 76%). This conclusion is also indirectly supported via the finding in chapter 4 that self-rated temperament yielded heritability estimates of 34% to 46%. This suggests that the low heritability of self-ratings is not specific to ADHD and may be a more general characteristic of twin research on psychopathological and/or behavioural ratings; however, the heritability of parental ratings of infant temperament generally falls within a similar range (Emde et al., 1992, Saudino et al., 2000, Smith et al., 2012).

Understanding the full impact of measurement error associated with the use of self-ratings is an important goal for future research. In the classical twin model measurement error is subsumed by the non-shared environmental (E) component of variance (Rijsdijk and Sham, 2002); thus it is not possible to disentangle error from genuine effects of the unique environment. This can be addressed in future research by taking steps to reduce measurement error prior to conducting twin analyses. For example, prior twin research into the cognitive performance variables included in chapter 6 estimated higher heritabilities when correcting for measurement error based on test-retest reliabilities (Kuntsi et al., 2006). A second approach is to use latent factors, combining information across multiple measures to reduce overall error (Bouchard Jr and Loehlin, 2001). The merits of the latter approach are demonstrated in chapter 3, where the strongest genetic influences were for the common latent factor that combined

parent, teacher and self-ratings of ADHD symptoms (84%). Similarly, research into cognitive performance has shown that he use of composite measures acts to reduce error and increase estimates of heritability (Kuntsi et al., 2006).

Another important step for future research is to conduct similar comparisons of parent, teacher and self-ratings for other psychopathological traits. This would determine whether rater differences in estimates of heritability are specific to ADHD or a more general feature of twin research. In particular, analyses of the emotional symptoms, conduct problems and peer relationships scales of the Strengths and Difficulties Questionnaire (SDQ, Goodman, 2001) in the TEDS sample would generate results directly comparable to those reported for the SDQ hyperactivity scale in this thesis. Analyses of ADHD ratings could also be extended to examine the heritability of interview-based assessments of ADHD, such as the Parental Account of Children's Symptoms (PACS) used to diagnose ADHD in the IMAGE sample in chapter 7. The PACS was administered to a subset of the TEDS twins at around age 10, making such research feasible in future. Similar research has already been conducted looking at antisocial behaviour, where the heritability of self-ratings was lowest (42%), with higher heritability estimates for interview ratings (61%) and even higher estimates for the ratings from parents (69%) and teachers (76%) (Arseneault et al., 2003).

8.4.2 Contrast effects

Contrast effects were found in two of the studies reported in this thesis, occurring when cross-twin within trait correlations for dizygotic (DZ) twin pairs were less than half the correlations for monozygotic (MZ) twin pairs in the presence of significantly greater variances for DZ than MZ twins. These effects have been reported previously for ADHD and appear to be a form of rater bias associated with the use of parental ratings of behaviour (e.g. Simonoff et al., 1998). It was therefore not surprising that contrast effects were found for parent-rated ADHD symptoms using the SDQ in chapter 3, but not for teacher or self-ratings of ADHD. Contrast effects were also found for parent ratings of hyperactivity-impulsivity and inattention symptoms in chapter 5. This result is in keeping with prior twin research from the same sample that identified contrast effects for symptoms of inattention, but not for symptoms of hyperactivity or

impulsivity when modelled as two separate domains (Thapar et al., 2000). One explanation for the difference between this study and the previous study is that use of a composite scale of hyperactivity-impulsivity could have led to contrast effects.

One unexpected finding was in chapter 7, where the pattern of twin correlations and variances for inattention symptom ratings also indicated possible contrast effects. This was unexpected because inattention was assessed using a composite of parent and teacher ratings. For consistency with prior analyses, and to simplify the mediation model fit to the data, contrast effects were not modelled. Nonetheless, the presence of potential contrast effects in the SAIL sample should be explored in future.

Interestingly, contrast effects were also observed for parent ratings of emotional lability in chapter 5, but only for males. Since the contrast effect is thought to occur when the behaviours of each twin from a pair are directly compared, this could reflect a sex difference in the manifestation of emotional lability symptoms. Specifically, boys may be more likely to externalise the symptoms of emotional lability, whereas girls may internalise their symptoms. This explanation has similarly been proposed elsewhere (Robison et al., 2008) and warrants consideration in future twin studies of emotional lability.

8.4.3 Genetic non-additivity and ADHD

A related question concerns genetic non-additivity. Like contrast effects, non-additive genetic influences are implicated on the basis of lower DZ than MZ cross-twin within-trait correlations, but with no significant differences in the phenotypic variances for MZ and DZ twins. Univariate analyses revealed significant non-additive genetic influences for self-ratings of ADHD symptoms using the SDQ in chapter 3, and for the combined parent-teacher ratings of inattention in chapter 6. For other phenotypes (parent ratings using the SDQ in chapter 3, parent ratings of hyperactivity-impulsivity and inattention in chapter 5), a model including contrast effects proved a better fit, as detailed above; while for others still (teacher ratings using the SDQ in chapter 3, self-ratings of hyperactivity-impulsivity and inattention in chapter 4, parent ratings of

hyperactivity-impulsivity in chapter 6) the best fitting model was a more parsimonious solution including only additive genetic and non-shared environmental influences. These results paint a conflicting picture of the extent of the non-additive genetic influences on ADHD.

Conversely, the multivariate analyses in chapters 3 and 5 both identified significant non-additive genetic influences for ADHD, even after accounting for contrast effects. In chapter 3, significant non-additive genetic influences were found for the common latent factor, accounting for roughly half the variance in the multi-rater view of ADHD-related behaviours. In chapter 5, significant non-additive genetic influences were found at the specific level for parent rated symptoms of hyperactivity-impulsivity and inattention. These results are of particular interest as multivariate models have greater power to detect variance components than univariate models (Schmitz et al., 1998). One implication, therefore, is that genetic non-additivity may account for a significant proportion of the phenotypic variance in symptoms of ADHD, but that there is insufficient power to detect this in univariate analyses. Indeed, a simulation study indicates that univariate analyses have low power to detect non-additive genetic effects even with large samples equivalent in size to TEDS, in addition to low power to detect non-additivity in the presence of contrast effects (Rietveld et al., 2003).

Genetic non-additivity could explain the polygenic results in chapter 7. In that chapter, a profile score was significantly associated with ADHD affection status in a sample of cases and pseudo-controls. The same signal was also associated with parent ratings of hyperactivity-impulsivity on the Conners' Parent Rating Scale - Revised (CPRS-R), and with teacher ratings of ADHD on the SDQ in an independent sample from the general population (TEDS). This is interesting, since both of these measures do not appear to be under non-additive genetic influence based on existing twin research. For example, twin modelling does not indicate non-additive genetic influences on parent ratings of hyperactivity-impulsivity or inattention using the CPRS-R in TEDS (Greven et al., 2011c), while meta-analysis identifies significant non-additive genetic influences for symptoms of inattention but not for hyperactivity-impulsivity when assessed in childhood and adolescence (Nikolas and Burt, 2010). Further, the analyses in chapter 3 identified non-additive genetic influences and/or contrast

effects for parent and self-ratings of ADHD symptoms using the SDQ, but not for teacher ratings. Therefore, it appears that the profile score generated in chapter 7 was associated only with ADHD symptom ratings that were free from non-additive genetic effects and/or contrast effects. However, as noted in the discussion of chapter 7, these associations would likely not have survived correction for multiple testing.

The pattern of results from chapter 7 replicates a separate polygenic study of the same phenotypes in TEDS, using the Genome-wide Complex Traits Analysis (GCTA) method (Trzaskowski et al., in press). In that study the only ADHD-related measures for which SNP-wide heritability (SNP-h^2) estimates could be obtained were hyperactivity-impulsivity rated using the CPRS-R (SNP-h^2 = 6%) and teacher ratings from the SDQ (SNP-h^2 = 5%), although these heritability estimates were weak and not significantly different from zero (see Table 7.1, chapter 7). Moreover, low, non-significant SNP-h^2 heritability estimates were obtained across a range of other behavioural phenotypes measured in the TEDS study. The study concluded that the most likely explanation for these results was that in most cases quantitative behavioural phenotypes were under greater non-additive genetic influence than was previously thought, since non-additivity cannot be detected based on polygenic analyses using the GCTA method to assess common alleles. Yet a significant SNP-h^2 estimate of 28% has been obtained in GCTA analysis of ADHD affection status (Cross-Disorder Group of the Psychiatric Genomics Consortium, in press), suggesting that the clinical disorder must be under a greater degree of additive genetic influence. This could reflect a qualitative distinction between the ADHD as a category and a continuum, or perhaps some form of rater effect (Trzaskowski et al., in press).

To further understand why non-additive genetic influences might be important with regard to ADHD symptoms, parallels can be drawn with recent genetic studies of Cloninger's temperament dimensions. The majority of twin analyses have found that these dimensions are primarily influenced by additive genetic effects (Ando et al., 2002, Ando et al., 2004, Gillespie et al., 2003, Heath et al., 1994, Heiman et al., 2003, Heiman et al., 2004, Stallings et al., 1996), including the research presented in chapter 4 of this thesis. Yet recent research has

demonstrated a greater role for non-additive genetic influences than was previously assumed. First, a twin-sibling study by Keller et al. (2005) found evidence of significant non-additive genetic influences across all temperament dimensions, a convincing result since the inclusion of non-twin siblings in analyses substantially increases the power to detect non-additive genetic effects (Rietveld et al., 2003). Second, the GCTA method estimated lower heritabilities than would be expected if temperament was additive genetic in origin (Verweij et al., 2012). The conclusions drawn on the basis of these studies were that additive genetic effects account for a relatively small proportion of the variance in Cloninger's temperament dimensions, whereas an accumulated mutation load consisting of mildly deleterious rare alleles and/or genetic dominance and epistasis (i.e. genetic non-additivity) accounts for much of the broad-sense heritability (Verweij et al., 2012). This is referred to as mutation-selection and suggests that polygenic genetic influences may operate within families, with a non-additive genetic load transmitted through successive generations. Theoretically, the same set of conclusions could apply to ADHD symptoms and could explain the results of polygenic research.

In summary, the pattern of results in this thesis is consistent with non-additive genetic influences on ADHD symptoms within the general population. This appears to be particularly true for inattention. The overarching implication is that classical twin studies may underestimate the non-additive genetic influences on ADHD symptoms, either by dropping the non-additive genetic component from models in favour of more parsimonious solutions, or due to low power to detect non-additivity alongside or instead of contrast effects (Rietveld et al., 2003). Future research should follow this up by conducting more rigorous tests of the non-additive genetic influences on ADHD. One approach is to use extensions of the classical twin method, such as the twin sibling model used to examine Cloninger's dimensions of temperament (Keller et al., 2005). However, an even stronger approach is to make use of the extended-twin family design (ETFD), which provides more accurate estimates of genetic non-additivity (Keller et al., 2010). This is possible due to the inclusion of multiple family members of different degrees of relatedness, enabling multiple parameters to be estimated simultaneously (e.g. D and C) without model over-identification. Greater attention should also be paid to non-additive genetic processes at the molecular

level, and one method that is now being applied is machine-learning, which can test for non-additive effects such as gene-gene interactions in the polygenic analyses of genome-wide data.

8.4.4 The role of the environment

The main focus of this thesis has been on genetic associations, simply because the majority of twin analyses estimated stronger genetic than environmental effects. Nonetheless, there were significant non-shared environmental effects in all analyses. As discussed above (section 8.4.1) some of this effect will reflect error, and it is for this reason that the non-shared environmental parameter cannot be dropped from twin models (Rijsdijk and Sham, 2002). However, some of this effect likely also reflects genuine environmental influences. It is also interesting to note that throughout this thesis there were no significant shared environmental influences on the varied measures ADHD.

The lack of shared-environmental effect is consistent with Plomin's hypothesis that the non-shared environment is generally more important in shaping an individual's development (Plomin et al., 2008). It is also in line with Burt's conclusion that ADHD is exempt from shared environmental influences (Burt, 2009, Nikolas and Burt, 2010). However, as noted by Wood and colleagues, shared environmental effects can be difficult to detect using the classical twin design if they occur alongside non-additive genetic effects (Wood et al., 2010b). As discussed in section 8.4.3, non-additivity seems to influence ADHD. It should be noted here that even for disorders or traits with very high heritabilities, the role of the environment can still be critical. Understanding the full extent of the environmental influences on ADHD is therefore particularly important, not only to better characterise the aetiology of the disorder but also to inform the environmental interventions for the treatment of ADHD. This can also be addressed via future research using extensions to the classical twin design (Keller et al., 2010) and by explicitly studying the environment, including gene-environment interplay (Rutter et al., 2006).

8.4.5 Sex effects

Throughout the chapters in this thesis there was a tendency for males to score significantly higher than females for mean symptoms of ADHD based on child and adolescent data. This is in contrast to a few prior population-based studies showing that rates of ADHD symptoms do not differ significantly across sex (Alloway et al., 2010, Biederman et al., 2005b), but is in line with the majority of clinical studies that report a higher prevalence of ADHD in males during childhood and adolescence (Gaub and Carlson, 1997, Gershon, 2002, Novik et al., 2006, Rucklidge, 2008). It is also in line with the results of prior twin research (Greven et al., 2011c, Larsson et al., 2006). Therefore the general trend across studies is for significantly higher levels of ADHD symptomatology in boys.

A potential explanation for this pattern of results is that the symptoms of ADHD may go undetected in girls, leading to mean differences in the ratings of symptoms of hyperactivity-impulsivity and inattention, and differences in sex ratios across clinics, but not necessarily reflecting genuine differences in the presence or absence of ADHD *per-se* (Staller and Faraone, 2006). This hypothesis is somewhat bolstered by the finding in chapter 6 that RTV scores were the same for boys and girls, suggesting that the one of the core cognitive deficits associated with ADHD did not differ as a function of sex. However, this argument assumes that RTV is an endophenotype for ADHD rather than simply being associated at a pleiotropic level. Furthermore, other cognitive performance variables, including MRT and commission errors, did show significant sex effects.

Another possible explanation for the sex difference is that girls may show greater levels of inattention and internalising symptoms, although research indicates that the prevalence of inattentive ADHD is typically higher among boys (Ford et al., 2003). Yet another potential explanation is one of rater effects, whereby different informants (e.g. parents or teachers) may tend to accentuate externalising behaviours among boys. However the research in chapter 3 indicated that higher mean scores among males were consistently found whether using parent, teacher or self-ratings of ADHD symptoms.

It should be noted that the one exception to this pattern of results was for the adult twin sample included in chapter 4, where females scored significantly higher than males for hyperactivity-impulsivity and with no sex differences in inattention. Although unexpected, a significantly higher level of ADHD symptoms among adult females has been reported in prior clinical research, perhaps reflecting greater levels of emotional symptoms and comorbidities (Robison et al., 2008). Mean harm avoidance in chapter 4 was significantly higher for females than males, suggestive of greater levels of emotionality; however the child and adolescent research presented in chapters 5 and 6 found significantly higher levels of emotional lability among males. This shift in symptoms could perhaps also reflect a developmental trend, whereby symptoms become more impairing in adult women than in men. This is also suggested in prior twin research, in which a gradual increase in the severity of symptoms in female relative to males has been found (Larsson et al., 2006).

Aetiological sex differences were examined across twin studies by fitting univariate full sex limitation models. A consistent finding was of variance sex differences for the symptoms of ADHD and emotional lability, with greater phenotypic variances found for males in line with the results of some prior twin research (e.g. Price et al., 2005). This was controlled for in analyses by constraining male variances to be a scalar multiple of the female variances. Again, the only exception to this rule was for the adults examined in chapter 4, where no significant variance sex differences were observed for ADHD symptoms, and where female variances were significantly greater for the temperament dimension of harm avoidance. None of the twin studies in this thesis found evidence of qualitative or quantitative sex differences, indicating that the aetiological influences on ADHD symptoms are the same across sex.

8.4.6 Heterogeneity of the ADHD phenotype

The twin research in chapters 4, 5 and 6 examined the two ADHD dimensions separately, consistently indicating substantial but imperfect phenotypic and genetic associations between hyperactivity-impulsivity and inattention. This is indicative of phenotypic and genetic heterogeneity and is in line with findings

from prior twin research (Greven et al., 2011c, Larsson et al., 2012b, McLoughlin et al., 2007). To an extent, the results reported in chapter 7 can be seen as demonstrating heterogeneity at the molecular genetic level, finding that a polygenic signal for ADHD affection status was associated with hyperactive-impulsive but not inattentive scores from the CPRS-R among the general population. As noted above (section 8.4.3), this finding could reflect greater non-additive genetic influences on the inattentive but not hyperactive-impulsive symptom dimension. Nonetheless, this result suggests a degree of separation in the genetic architecture of the hyperactive-impulsive and inattentive domains.

The differential association of hyperactivity-impulsivity and inattention with co-occurring traits is further evidence of heterogeneity in ADHD. In chapter 4, the temperament dimension of novelty seeking was associated with both dimensions of ADHD, whereas harm avoidance was uniquely associated with inattention, and persistence was positively associated with hyperactivity-impulsivity and negatively associated with inattention. In chapters 5 and 6, emotional lability was associated with both ADHD dimensions, but significantly more strongly with hyperactivity-impulsivity than inattention. This was most apparent in the mediation modelling conducted in chapter 6, where there was a substantial unique association between emotional lability and hyperactivity-impulsivity. In contrast, RTV appeared more strongly related to inattention. This potentially highlights a separation of externalised behaviours (hyperactivity, impulsivity, emotional lability) from attention-related traits (inattention, RTV).

The finding of heterogeneity is important for at least two reasons. First, heterogeneity is one explanation for the missing heritability in molecular genetic research and could account for the modest polygenic associations found in chapter 7 (Manolio et al., 2009). Future research should therefore aim to identify genetically homogeneous subpopulations for inclusion in molecular analyses, to see whether this improves the power to detect genetic associations for ADHD. Second, identifying more homogeneous subpopulations may be of benefit to clinical practice, since sub-groups of individuals with ADHD might differ in terms of symptom presentation, comorbidity, functional impairments, underlying neurobiology and treatment response.

One option for conducting such research is to take forward the results from chapter 4, for example by examining whether individuals with ADHD who are high versus low in harm avoidance differ in terms of their phenotypic and clinical presentations or in terms of associations with different sets of genes. The former of these approaches has already been tested using the five-factor model of personality, with evidence that different profiles of temperament can be used to characterise distinct profiles or subtypes of ADHD (Martel et al., 2011, Nigg et al., 2004b). The latter has already been tested via candidate gene research, with evidence that different risk alleles were associated with distinct profiles of temperament in a clinical sample of adults with ADHD (de Cerqueira et al., 2011). Future research should not only build on these results but should also take a longitudinal perspective, examining the developmental trajectories of different personality profiles over time.

8.4.7 ADHD and emotional lability

The aetiological relationship between ADHD and emotional lability was demonstrated across three different studies in chapters 5, 6 and 7. The first identified shared genetic influences for hyperactivity-impulsivity, inattention and emotional lability in child and adolescent twins; the second confirmed the genetic association in a separate child twin sample but revealed no direct relationship between emotional lability and cognitive performance; the third provided tentative evidence of association at the molecular genetic level, also in children.

A number of studies have previously demonstrated an association between ADHD and emotional lability in clinical populations, including evidence of concomitant treatment effects, as well as the strong clinical association of emotional lability even in non-comorbid ADHD cases, leading to the hypothesis that emotional lability might be seen as core component of ADHD (Barkley, 2010, Corbisiero et al., 2013, Retz et al., 2012, Skirrow and Asherson, 2013, Skirrow et al., 2009). Indeed, this view is expressed in DSM-5 where the presence of emotional lability is listed as supporting evidence for the diagnosis of ADHD. While the results across chapters 5-7 are not unequivocal, the consistent evidence of genetic associations indicates a substantial overlap in

the liability for emotional lability and ADHD. The results of chapters 5 and 6 additionally show that, at the phenotypic level, hyperactivity-impulsivity is as strongly related to emotional lability as it is to inattention. If one took the view that hyperactivity-impulsivity represents the central deficit in ADHD, then one could argue that emotional lability and inattention are equally important components of the broader ADHD phenotype.

Further research will be required to evaluate the full extent of the association between ADHD and emotional lability. As discussed above (section 8.3.1), an important first step will be to establish factorial independence of ADHD and emotional lability from similar phenotypes, including oppositional defiant disorder (ODD), bipolar disorder and depression. The need to examine ODD is particularly pressing, since it also features symptoms of emotional lability (Ezpeleta et al., 2012, Kuny et al., 2013, Rowe et al., 2010, Stringaris and Goodman, 2009b) and since it also shows strong genetic associations with the symptoms of hyperactivity-impulsivity in twin research (Wood et al., 2009a). This will help to determine how and why an externalising spectrum of disorders co-occur during childhood.

Another step will be to examine the longitudinal associations between ADHD and emotional lability using developmental and genetically-sensitive designs. This is particularly important given the results of *ad-hoc* analyses in chapter 5, which showed that the genetic association between ADHD and emotional lability was stronger in older than younger individuals. One project examining the phenotypic associations over the course of development is already underway and is also examining how ADHD and emotional lability are related to depression across the lifespan (Ryckaert, unpublished data). However, additional quantitative genetic studies are required; first to determine the aetiological relationship between ADHD and emotional lability in adults; then to examine stability and change in the genetic and environmental associations across the lifespan. Other studies should also seek to establish the molecular genetic basis of the relationship between hyperactivity-impulsivity, inattention and emotional lability, and should search for cognitive measures that index the common liability across these traits.

8.5 CLINICAL IMPLICATIONS

8.5.1 Assessment of ADHD

The twin research in chapter 3 suggested higher reliability for composite ratings of ADHD than for individual parent, teacher or self-ratings. Therefore, one implication is that self-ratings of ADHD symptoms should be routinely collected throughout childhood and adolescence, alongside informant reports. This may provide a more accurate clinical picture than simply relying on informant-ratings alone. Similarly the results in chapters 3 and 4 suggest that adult clinics, which typically rely on self-reports (Asherson, 2005), should make increasing use of other informant data where possible.

8.5.2 Understanding the aetiology of ADHD

Understanding why ADHD occurs is important for clinical practice for several reasons. First, information on the aetiology of ADHD informs clinical management and treatment of the disorder. Second, psycho-education regarding the causes and course of ADHD is recommended as part of the care pathway for those diagnosed (NICE, 2008). Third, greater understanding of aetiological factors has the potential to reduce stigma, providing further evidence that ADHD is a neurodevelopmental disorder and not simply a problem in childhood caused by poor parental discipline (Mayes et al., 2008). The twin research in this thesis builds on a wealth of previous studies to suggest that ADHD is substantially influenced by genetic factors. In addition, the polygenic results presented in chapter 7 indicate a molecular genetic basis for ADHD as a clinical disorder and as a quantitative trait.

8.5.3 Recognising related phenotypes

An understanding of the aetiological association between ADHD and related traits is similarly important to clinical practice. The twin research in chapters 5 and 6 indicates that a substantial amount of the genetic liability between hyperactivity-impulsivity, inattention and emotional lability is shared, a finding that is somewhat replicated at the polygenic level in chapter 7. The results in

chapter 4 similarly suggest that distinct temperamental profiles may characterise different subtypes of ADHD. Clinicians should be aware of the genetic association between ADHD and these related traits when seeing patients. In particular, the results from chapters 5 to 7 suggest that professionals working in child and adolescent mental health services (CAMHS) should be mindful that patients with ADHD are likely to experience symptoms of emotional lability, and that patients presenting with labile, volatile moods may have untreated symptoms of ADHD.

8.5.4 Treating ADHD and emotional lability

Because of the shared aetiology demonstrated in chapters 5, 6 and 7, co-occurring symptoms of ADHD and emotional lability should form a target for treatment. A wealth of studies suggest that emotional lability responds well to both stimulant and atomoxetine medication in adults (Marchant et al., 2011a, Marchant et al., 2011b, Reimherr et al., 2005b, Reimherr et al., 2007, Rosler et al., 2010), although such a treatment effect in childhood and adolescence is yet to be fully established. Examining medication effects on emotional lability in childhood and adolescence should be a goal of future clinical research, while in the meantime it is recommended that clinicians monitor the effects of medication on emotional lability symptoms when prescribing to this age group.

Although the results of this thesis suggest that the association between ADHD and emotional lability is primarily genetic in origin, this does not preclude the use of non-pharmacological interventions to treat emotional lability symptoms. In childhood and early adolescence, emotional lability can be addressed via parent training programmes, recommended for the treatment of a range of emotional and behavioural problems in the UK (NICE, 2008, NICE, 2013). Other efficacious interventions for child and adolescent ADHD have yet to be trialed for the treatment of emotional lability, including dietary restriction, fatty acid supplementation, neuro-feedback and cognitive training (Sonuga-Barke et al., 2013).

In later adolescence and adulthood, the symptoms of emotional lability may be treated via cognitive therapies delivered at the individual or group level. The

Young-Bramham programme of cognitive behavioural therapy (CBT) for adult ADHD includes a module on self-regulation (Young and Bramham, 2012), while the R and R2 programme delivers CBT to address ADHD and comorbid antisocial behaviour problems including emotion regulation (Young and Ross, 2007). Mindfulness-based therapy has also been found to improve self-regulation in ADHD (Zylowska et al., 2008), while dialectical behaviour therapy (DBT) might also be beneficial. DBT combines CBT with elements of mindfulness and acceptance therapies. It was originally developed for the treatment of borderline personality disorder, which itself features symptoms of emotional instability (Fossati et al., 2002), and there is emerging evidence that DBT alleviates such symptoms in ADHD (Philipsen et al., 2007).

8.5.5 A positive perspective

The clinical implications of the research presented in this thesis are predominantly negative, in so far as research has focused on aetiological associations between ADHD and a range of cognitive and behavioural deficits. The deficit-based model of ADHD highlights the chronic and impairing nature of severe inattentive, hyperactive and impulsive symptoms. However, it is often important for clinicians to identify the strengths, as well as weaknesses that characterise individual patients. This is exemplified in the Young-Bramham CBT programme for adolescent and adult ADHD, which concludes with a module on individual strengths to engender resilience and hope for the future (Young and Bramham, 2012). This is consistent with a model of positive psychology (Seligman and Csikszentmihalyi, 2000), which could be applied to interpret some of the research in this thesis. One example is the research in chapter 4, which identified genetic associations between ADHD and novelty seeking. While novelty seeking is associated with a range of impairments, such as substance misuse (Wills et al., 1998), there are also putative links to positive psychological traits like creativity (Schweizer, 2006). The positive impact of being high in novelty seeking is something that clinicians could emphasise when working to improve efficacy, self-esteem and resilience among patients with ADHD, although further empirical research in this field is ultimately required.

8.6 FUTURE DIRECTIONS

8.6.1 Further research

A number of future directions for research have been proposed in this discussion. Yet three major themes emerge. First, future studies should seek to clarify the full extent of non-additive genetic and environmental influences on ADHD, including via the extended-twin family design. Several twin registers already include data from extended pedigrees (e.g. the Swedish Twin Study of Child and Adolescent Development, Lichtenstein et al., 2007) and could make use of this in future analyses of ADHD-related traits. This will have important consequences in guiding molecular genetic research. Further research using the GCTA method will also help to clarify the importance of additive versus non-additive genetic influences at the molecular level. Second, future studies should examine whether more homogeneous subtypes of ADHD can be identified, particularly on the basis of profiles of temperament. One important method will be to examine the developmental trajectories of children with different profiles of temperament. This research will likely impact future genetic studies and has the potential to inform clinical practice if clinically meaningful temperament profiles are found. Third, further aetiological research is required to understand how and why emotional lability is associated with ADHD. The most pressing concern is to conduct factor analyses to unpick the associations between ADHD, oppositional defiance and emotional lability, before moving on to study common aetiological influences across development. The aetiological component of research should not only focus on genetics, but also on identifying cognitive and neurobiological markers for emotional lability in ADHD.

8.6.2 Personal goals

The research presented in this thesis was conducted due to an initial enthusiasm to study ADHD that developed into a fervent interest. Accordingly, a short-term personal goal is to conduct some of the follow-up analyses recommended herein. For example, analysis of emotional lability and ADHD symptoms in adult twins is currently underway (Merwood, Larsson, Rijsdijk, Chen and Asherson), as is a systematic review of the genetic associations

between ADHD and Cloninger's dimensions of temperament (Merwood, Nijjar and Asherson). A twin study is also planned to examine the relationship between novelty seeking, emotional lability and hyperactivity-impulsivity in childhood (Merwood, Rijsdijk, Kuntsi and Asherson). The polygenic analyses reported in chapter 7 are also being followed up and the results have been already been used to guide a machine learning project examining the genetic basis of ADHD (Malki, Merwood, Neale, Faraone, Kuntsi and Asherson, on behalf of the ADHD subgroup of the PGC). Follow-up analyses using the IMAGE sample are also planned.

A long-term personal goal is to integrate research into ADHD with clinical practice and to develop the skills required for a career as a clinical academic. This goal will be achieved by training in clinical psychology, including undertaking specialist placements focused on the treatment of child, adolescent and adult ADHD. This training will also include research, and it is envisaged that this some of this research will include empirical studies of the potentially advantageous aspects of ADHD.

8.7 CONCLUSION

In conclusion, this thesis has presented novel research findings regarding the aetiology of ADHD and its association with co-occurring traits. The pitfalls of different informant ratings of ADHD were evaluated; the phenotypic and genetic associations with temperament discovered; the common aetiology of ADHD and emotional lability established; and the polygenic basis of ADHD confirmed. These results pave the way for future studies into ADHD and have the potential to inform several aspects of clinical practice.

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APPENDICES

APPENDIX A

Tables A1-3 provide fit statistics for the univariate sex-limited models reported in chapter 3. All tables include the following statistics: -2LL = log likelihood statistic, df = degrees of freedom, AIC = Akaike's Information Criterion; BIC = Bayesian Information Criterion; $\chi 2$ = likelihood ratio test (LRT) for difference between full and restricted models; Δdf = difference in degrees of freedom for LRT; *p* = significance of LRT. The best-fitting models are denoted in **bold**.

Full sex limitation models allowed quantitative and qualitative sex differences, with either r_A or r_D between twin 1 and twin 2 set to vary freely; Common sex limitation models allowed quantitative sex differences but not qualitative differences; Scalar sex limitation models allowed variance differences between males and females and females but no qualitative or quantitative differences; the null model equated all variance parameters to be equal across sex. Full details of the sex limitation model are provided in section 2.3.6. Contrast effects (*b*) were initially parameterised separately for male, female and opposite-sex twin pairs. They were then equated across sex to see whether this led to a significant deterioration in model fit, as a test of sex differences.

Table A1: Fit statistics for univariate modelling of **parent ratings ADHD**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	16475.21	11153	-5830.79	-39880.53	-	-	-
ADE Full (r_A free)	16548.88	11169	-5789.12	-39912.73	-	-	-
ADE Full (r_D free)	16548.88	11169	-5789.12	-39912.73	-	-	-
ADE Common	16548.88	11170	-5791.12	-39917.04	0.00	1	1.00
ADE Scalar	16551.78	11172	-5792.22	-39924.22	2.90	3	0.41
ADE Null	16598.77	11173	-5747.23	-39905.04	49.89	4	<.001
AE Scalar	16618.16	11173	-5727.84	-39895.34	69.28	4	<.001
ADE-*b* Full (r_A free)	16534.33	11166	-5797.66	-39907.06	-	-	-
ADE-*b* Full (r_D free)	16534.33	11166	-5797.66	-39907.06	-	-	-
ADE-*b* Common	16534.33	11167	-5799.66	-39911.37	0.00	1	1.00
ADE-*b* Scalar	16539.72	11169	-5798.28	-39917.31	5.39	3	0.15
ADE-*b* Null	16585.10	11170	-5754.90	-39898.93	50.77	4	<.001
AE-*b* Scalar	16539.72	11170	-5800.28	-39921.62	5.39	4	0.25
AE-*b* Scalar [A]	**16541.35**	**11172**	-5802.65	**-39929.43**	**7.02**	**6**	**0.32**

[A] Denotes that the rater contrast effect was equated (eq.) for males and females.

Table A2: Fit statistics for univariate modelling of **teacher ratings ADHD**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	16468.48	9340	-2211.52	-31739.46	-	-	-
ADE Full (r_A free)	16488.27	9356	-2223.73	-31798.04	-	-	-
ADE Full (r_D free)	16488.89	9356	-2223.11	-31797.73	-	-	-
ADE Common	16488.27	9357	-2225.73	-31802.32	0.00	1	1.00
ADE Scalar	16493.34	9359	-2224.66	-31808.33	5.07	3	0.17
ADE Null	16732.23	9360	-1987.77	-31693.18	243.96	4	<.001
AE Scalar	**16493.37**	**9360**	**-2226.63**	**-31812.61**	**5.10**	**4**	**0.28**

Table A3: Fit statistics for univariate modelling of **child self-ratings**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	17764.16	11133	-4501.84	-39180.56	-	-	-
ADE Full (r_A free)	17810.62	11149	-4487.38	-39226.40	-	-	-
ADE Full (r_D free)	17810.62	11149	-4487.38	39226.40	-	-	-
ADE Common	17811.28	11150	-4488.72	-39230.39	0.66	1	.418
ADE Scalar	**17812.97**	**11152**	-4491.03	**-39238.18**	**2.35**	**3**	**.504**
ADE Null	17821.18	11153	-4484.82	-39238.39	10.56	4	.032
AE Scalar	17821.08	11153	-4484.92	-39238.44	10.46	4	.033

Table A4: Fit statistics for univariate modelling of **same-teacher ratings**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	5903.11	3695	-1486.89	-10964.97	-	-	-
ADE Full (r_A free)	5933.36	3711	-1488.64	-11010.10	-	-	-
ADE Full (r_D free)	5933.36	3711	-1488.64	-11010.10	-	-	-
ADE Common	5933.36	3712	-1490.64	-11013.87	0	1	1.00
ADE Scalar	5935.16	3714	-1492.84	-11020.50	1.79	3	0.62
ADE Null	6068.11	3715	-1361.89	-10957.79	134.75	4	<.001
AE Scalar	**5935.82**	**3715**	-1494.18	**-11023.94**	**2.46**	**4**	**0.65**

Table A5: Fit statistics for univariate modelling of **different-teacher ratings**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	10377.25	5620	-862.75	-17618.51	-	-	-
ADE Full (r_A free)	10387.64	5636	-884.36	-17678.24	-	-	-
ADE Full (r_D free)	10387.64	5636	-884.36	-17678.24	-	-	-
ADE Common	10387.34	5637	-886.36	-17682.30	0	1	1.00
ADE Scalar	10393.43	5639	-884.57	-17687.52	5.79	3	0.12
ADE Null	10508.43	5640	-771.57	-17634.08	120.79	4	<.001
AE Scalar	**10393.43**	**5640**	-886.57	**-17691.58**	**5.79**	**4**	**0.22**

APPENDIX B

Tables B1-6 provide fit statistics for the univariate sex-limited models reported in chapter 4. All tables include the following statistics: -2LL = log likelihood statistic, df = degrees of freedom, AIC = Akaike's Information Criterion; BIC = Bayesian Information Criterion; $\chi 2$ = likelihood ratio test (LRT) for difference between full and restricted models; Δdf = difference in degrees of freedom for LRT; *p* = significance of LRT. The best-fitting models are denoted in **bold**.

Full sex limitation models allowed quantitative and qualitative sex differences, with either r_A or r_D or r_C between twin 1 and twin 2 set to vary freely; Common sex limitation models allowed quantitative sex differences but not qualitative differences; Scalar sex limitation models allowed variance differences between males and females and females but no qualitative or quantitative differences; the null model equated all variance parameters to be equal across sex. Details of the sex limitation model are provided in section 2.3.6.

Table A1: Fit statistics for univariate modelling of **hyperactivity-impulsivity**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	3108.99	1609	-109.02	-3974.97	-	-	-
ADE Full (r_A free)	3139.28	1625	-110.72	-4014.81	-	-	-
ADE Full (r_D free)	3140.15	1625	-109.85	-4014.37	-	-	-
ADE Common	3140.15	1626	-111.85	-4017.81	0.86	1.00	0.35
ADE Scalar	3142.75	1628	-113.25	-4023.38	3.47	3.00	0.32
ADE Null	3142.90	1629	-115.10	-4026.74	3.62	4.00	0.46
AE Null	3142.93	1630	-117.07	-4030.16	3.65	5.00	0.60
ACE Full (r_A free)	3136.46	1625	-113.54	-4016.22	-	-	-
ACE Full (r_C free)	3136.54	1625	-113.46	-4016.18	-	-	-
ACE Common	3136.54	1626	-115.46	-4019.61	0.08	1.00	0.78
ACE Scalar	3142.79	1628	-113.21	-4023.36	6.33	3.00	0.10
ACE Null	3142.93	1629	-115.07	-4026.73	6.47	4.00	0.17
AE Null	3142.93	1630	-117.07	-4030.16	6.47	5.00	0.26
CE Null	3151.44	1630	-108.56	-4025.91	14.98	5.00	<.05

Table B2: Fit statistics for univariate modelling of **inattention**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	2688.02	1596	-503.98	-4137.46	-	-	-
ADE Full (r_A free)	2718.29	1612	-505.71	-4177.28	-	-	-
ADE Full (r_D free)	2718.29	1612	-505.71	-4177.28	-	-	-
ADE Common	2718.29	1613	-507.71	-4180.72	0.00	1.00	1.00
ADE Scalar	2719.00	1615	-511.00	-4187.23	0.71	3.00	0.87
ADE Null	2719.23	1616	-512.77	-4190.55	0.94	4.00	0.92
AE Null	**2719.47**	**1617**	**-514.53**	**-4193.86**	**1.18**	**5.00**	**0.95**
ACE Full (r_A free)	2719.01	1612	-504.99	-4176.92	-	-	-
ACE Full (r_C free)	2719.01	1612	-504.99	-4176.92	-	-	-
ACE Common	2719.01	1613	-506.99	-4180.35	0.00	1.00	1.00
ACE Scalar	2719.22	1615	-510.79	-4187.12	0.20	3.00	0.98
ACE Null	2719.47	1616	-512.53	-4190.43	0.46	4.00	0.98
AE Null	2719.47	1617	-514.53	-4193.86	0.46	5.00	0.99
CE Null	2728.13	1617	-505.87	-4189.53	9.12	5.00	0.10

Table B3: Fit statistics for univariate modelling of **novelty seeking**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	8454.06	1574	5306.06	-1171.49	-	-	-
ADE Full (r_A free)	8480.33	1590	5300.33	-1213.23	-	-	-
ADE Full (r_D free)	8480.13	1590	5300.13	-1213.33	-	-	-
ADE Common	8480.33	1591	5298.33	-1216.66	0.00	1.00	1.00
ADE Scalar	8480.58	1593	5294.58	-1223.40	0.25	3.00	0.97
ADE Null	**8481.38**	**1594**	**5293.38**	**-1226.42**	**1.05**	**4.00**	**0.90**
AE Null	8491.57	1595	5301.57	-1224.76	11.24	5.00	<.05
ACE Full (r_A free)	8487.60	1590	5307.60	-1209.59	-	-	-
ACE Full (r_C free)	8489.93	1590	5309.93	-1208.43	-	-	-
ACE Common	8489.93	1591	5307.93	-1211.86	2.33	1.00	0.13
ACE Scalar	8490.83	1593	5304.83	-1218.27	3.23	3.00	0.36
ACE Null	8491.57	1594	5303.57	-1221.33	3.97	4.00	0.41
AE Null	8491.57	1595	5301.57	-1224.76	3.97	5.00	0.55
CE Null	8515.15	1595	5325.15	-1212.97	27.55	5.00	<.001

Table B4: Fit statistics for univariate modelling of **harm avoidance**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	9178.47	1578	6022.47	-824.66	-	-	-
ADE Full (r_A free)	9190.42	1594	6002.42	-873.58	-	-	-
ADE Full (r_D free)	9190.42	1594	6002.42	-873.58	-	-	-
ADE Common	9190.42	1595	6000.42	-877.01	0.00	1.00	1.00
ADE Scalar	9191.55	1597	5997.55	-883.30	1.13	3.00	0.77
ADE Null	9200.77	1598	6004.77	-882.13	10.35	4.00	<.05
AE Scalar	**9202.58**	**1599**	**6004.58**	**-884.65**	**2.87**	**4.00**	**0.58**
ACE Full (*r*A free)	9191.67	1594	6003.67	-872.95	-	-	-
ACE Full (*r*C free)	9192.01	1594	6004.01	-872.78	-	-	-
ACE Common	9192.01	1595	6002.01	-876.21	0.35	1.00	0.55
ACE Scalar	9193.29	1597	5999.29	-882.43	1.62	3.00	0.66
ACE Null	9202.58	1598	6006.58	-881.22	10.92	4.00	<.05
AE Scalar	9202.58	1599	6004.58	-884.65	1.62	4.00	0.81
CE Scalar	9222.59	1599	6024.59	-874.64	21.12	4.00	<.001

Table B5: Fit statistics for univariate modelling of **reward dependence**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	7304.62	1555	4194.62	-1676.13	-	-	-
ADE Full (r_A free)	7315.93	1571	4173.93	-1725.30	-	-	-
ADE Full (r_D free)	7315.90	1571	4173.90	-1725.31	-	-	-
ADE Common	7315.93	1572	4171.93	-1728.73	0.00	1.00	1.00
ADE Scalar	7316.12	1574	4168.12	-1735.49	0.19	3.00	0.98
ADE Null	7316.12	1575	4166.12	-1738.91	0.19	4.00	1.00
AE Null	**7321.99**	**1576**	**4169.99**	**-1739.41**	**6.06**	**5.00**	**0.30**
ACE Full (r_A free)	7319.39	1571	4177.39	-1723.57	-	-	-
ACE Full (r_C free)	7321.10	1571	4179.10	-1722.72	-	-	-
ACE Common	7321.10	1572	4177.10	-1726.15	1.70	1.00	0.19
ACE Scalar	7321.99	1574	4173.99	-1732.55	2.60	3.00	0.46
ACE Null	7321.99	1575	4171.99	-1735.98	2.60	4.00	0.63
AE Null	7321.99	1576	4169.99	-1739.41	2.60	5.00	0.76
CE Null	7337.99	1576	4185.99	-1731.41	18.59	5.00	<.01

Table B6: Fit statistics for univariate modelling of **persistence**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	5851.10	1554	2743.10	-2402.74	-	-	-
ADE Full (r_A free)	5863.51	1570	2723.51	-2451.39	-	-	-
ADE Full (r_D free)	5863.51	1570	2723.51	-2451.39	-	-	-
ADE Common	5863.51	1571	2721.51	-2454.82	0.00	1.00	1.00
ADE Scalar	5863.63	1573	2717.63	-2461.62	0.12	3.00	0.99
ADE Null	5865.82	1574	2717.82	-2463.96	2.30	4.00	0.68
AE Null	**5865.82**	**1575**	**2715.82**	**-2467.38**	**2.30**	**5.00**	**0.81**
ACE Full (r_A free)	5863.30	1570	2723.30	-2451.50	-	-	-
ACE Full (r_C free)	5863.30	1570	2723.30	-2451.50	-	-	-
ACE Common	5863.30	1571	2721.30	-2454.93	0.00	1.00	1.00
ACE Scalar	5863.52	1573	2717.52	-2461.68	0.21	3.00	0.98
ACE Null	5865.73	1574	2717.73	-2464.00	2.45	4.00	0.65
AE Null	5865.82	1575	2715.82	-2467.38	2.52	5.00	0.77
CE Null	5870.04	1575	2720.04	-2465.27	6.73	5.00	0.24

APPENDIX C

Tables C1-3 provide fit statistics for the univariate sex-limited models reported in chapter 5. All tables include the following statistics: -2LL = log likelihood statistic, df = degrees of freedom, AIC = Akaike's Information Criterion; BIC = Bayesian Information Criterion; χ2 = likelihood ratio test (LRT) for difference between full and restricted models; ∆df = difference in degrees of freedom for LRT; *p* = significance of LRT. The best-fitting models are denoted in **bold**.

Full sex limitation models allowed quantitative and qualitative sex differences, with either r_A or r_D between twin 1 and twin 2 set to vary freely; Common sex limitation models allowed quantitative sex differences but not qualitative differences; Scalar sex limitation models allowed variance differences between males and females and females but no qualitative or quantitative differences; the null model equated all variance parameters to be equal across sex. Details of the sex limitation model are provided in section 2.3.6. Where included, contrast effects (*b*) were initially parameterised separately for male, female and opposite-sex twin pairs. They were then equated across sex to see whether this led to a significant deterioration in model fit, as a test of sex differences. The decision on whether to model ADE, ADE-*b*, ACE, or a hybrid model was based on the pattern of twin variances and correlations.

Table C1: Fit statistics for univariate modelling of **hyperactivity-impulsivity**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	10349.42	3815	2719.42	-9246.14	-	-	-
ADE Full (r_A free)	10363.77	3831	2701.77	-9299.45	-	-	-
ADE Full (r_D free)	10363.77	3831	2701.77	-9299.45	-	-	-
ADE Common	10363.97	3832	2699.97	-9303.13	0.20	1	0.65
ADE Scalar	10370.24	3834	2702.24	-9307.56	6.47	3	0.09
ADE Null	10399.75	3835	2729.75	-9296.58	35.98	4	<.001
AE Scalar	10410.28	3835	2740.28	-9291.32	46.50	4	<.001
ADE-*b* Full (r_A free)	10355.84	3827	2701.84	-9288.30	-	-	-
ADE-*b* Full (r_D free)	10355.84	3827	2701.84	-4014.37	-	-	-
ADE-*b* Common	10355.84	3828	2699.84	-9292.08	0.00	1	1.00
ADE-*b* Scalar	10358.12	3830	2698.12	-9298.49	2.28	3	0.52
ADE-*b* Null	10369.98	3831	2707.98	-9296.34	14.15	4	<.05
AE-*b* Scalar	10358.12	3831	2696.12	-9302.27	2.28	4	0.68
AE-*b* Scalar [A]	**10364.80**	**3834**	**2696.80**	**-9310.27**	**8.96**	**7**	**0.26**

[A] Denotes that the rater contrast effect was equated (eq.) for males and females.

Table C2: Fit statistics for univariate modelling of **inattention**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	11121.23	3815	3491.23	-8860.24	-	-	-
ADE Full (r_A free)	11142.09	3831	3480.09	-8910.29	-	-	-
ADE Full (r_D free)	11142.09	3831	3480.09	-8910.29	-	-	-
ADE Common	11142.09	3832	3478.09	-8914.07	0.00	1	1.00
ADE Scalar	11144.82	3834	3476.82	-8920.26	2.73	3	0.43
ADE Null	11160.65	3835	3490.65	-8916.13	18.56	4	<.001
AE Scalar	11176.52	3835	3506.52	-8908.20	34.43	4	<.001
ADE-*b* Full (r_A free)	11134.52	3827	3480.52	-8898.96	-	-	-
ADE-*b* Full (r_D free)	11134.56	3827	3480.56	-8898.94	-	-	-
ADE-*b* Common	11134.56	3828	3478.56	-8902.72	0.04	1	0.84
ADE-*b* Scalar	11136.56	3830	3476.56	-8909.27	2.04	3	0.56
ADE-*b* Null	11146.11	3831	3484.12	-8908.28	11.59	4	<.05
AE-*b* Scalar	11136.56	3831	3474.56	-8913.06	2.04	4	0.73
AE-*b* Scalar [A]	**11136.91**	**3834**	**3468.91**	**-8924.22**	**2.40**	**7**	**0.93**

[A] Denotes that the rater contrast effect was equated (eq.) for males and females.

Table C3: Fit statistics for univariate modelling of **emotional lability**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	7492.87	3815	-137.14	-10674.42	-	-	-
ACDE-*b* Full (r_A free) [A]	7507.09	3830	-152.91	-10724.01	-	-	-
AE-*b* Full (r_A free) [B]	7507.49	3832	-156.51	-10731.37	0.40	2	0.82
AE-*b* Common	7514.99	3833	-151.02	-10731.40	7.90	3	<.05
AE-*b* Scalar	**7515.30**	**3834**	**-152.70**	**-10735.02**	**8.21**	**4**	**0.08**
AE-*b* Null	7529.69	3835	-140.31	-10731.61	22.19	5	<.001
AE Scalar	7523.65	3835	-146.35	-10734.63	11.53	5	<.05

[A] Denotes hybrid model, including C for females, and D and -*b* for males.

[B] To enable tests of whether genetic/environmental parameters were the same for males and females, C and D were dropped from the full sex-limitation model. This did not result in a significant deterioration in fit, thus all subsequent sex-limitation models parameterised AE-*b*, with the contrast effect (*b*) for males only.

APPENDIX D

Tables D1-4 provide fit statistics for the univariate sex-limited models reported in chapter 6. All tables include the following statistics: -2LL = log likelihood statistic, df = degrees of freedom, AIC = Akaike's Information Criterion; BIC = Bayesian Information Criterion; χ2 = likelihood ratio test (LRT) for difference between full and restricted models; ∆df = difference in degrees of freedom for LRT; *p* = significance of LRT. The best-fitting models are denoted in **bold**.

Full sex limitation models included either ADE or ACE, depending on the pattern of twin correlations (Table 6.3). The full model allowed quantitative and qualitative sex differences, with either r_A or r_D or r_C between twin 1 and twin 2 set to vary freely; Common sex limitation models allowed quantitative sex differences but not qualitative differences; Scalar sex limitation models allowed variance differences between males and females and females but no qualitative or quantitative differences; the null model equated all variance parameters to be equal across sex. Details of the sex limitation model are provided in section 2.3.6.

Tables D5-6 give the formulas used to calculate standardised solutions of the mediation models presented in chapter 6. The unstandardised residuals used in these calculations are presented in Table 6.7, chapter 6.

Table D1: Fit statistics for univariate modelling of **hyperactivity-impulsivity**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	5666.09	1134	3398.09	-804.32	-	-	-
ADE Full (r_A free)	5677.73	1150	3377.73	-849.81	-	-	-
ADE Full (r_D free)	5677.73	1150	3377.73	-849.81	-	-	-
ADE Common	5677.73	1151	3375.73	-853.02	0	1	1.00
ADE Scalar	5680.70	1153	3374.70	-857.95	2.97	3	0.40
ADE Null	5815.73	1154	3509.73	-790.44	138	4	<.001
AE Scalar	**5682.43**	**1154**	**3374.43**	**-860.30**	**4.70**	**4**	**0.32**

Table D2: Fit statistics for univariate modelling of **inattention**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	5769.97	1134	3501.97	-752.38	-	-	-
ADE Full (r_A free)	5794.20	1150	3494.20	-791.58	-	-	-
ADE Full (r_D free)	5793.87	1150	3493.87	-791.74	-	-	-
ADE Common	5794.20	1151	3492.20	-794.79	0.00	1	1.00
ADE Scalar	**5794.69**	**1153**	**3488.69**	**-800.96**	**0.49**	**3**	**0.92**
ADE Null	5902.89	1154	3596.89	-746.86	108.69	4	<.001
AE Scalar	5813.29	1154	3505.29	-794.87	19.09	4	<.001

Table D3: Fit statistics for univariate modelling of **emotional lability**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	5787.67	1130	3527.67	-728.84	-	-	-
ADE Full (r_A free)	5801.13	1146	3509.13	-773.41	-	-	-
ADE Full (r_D free)	5801.13	1146	3509.13	-773.41	-	-	-
ADE Common	5801.13	1147	3507.12	-776.61	0.00	1	1.00
ADE Scalar	5802.00	1149	3504.00	-782.59	0.87	3	0.83
ADE Null	5834.72	1150	3536.72	-766.23	33.59	4	<.001
AE Scalar	**5802.22**	**1150**	**3502.22**	-785.68	**1.09**	**4**	**0.90**

Table D4: Fit statistics for univariate modelling of **reaction time variability**

Model	-2LL	df	AIC	BIC	χ^2	Δdf	*p*
Saturated model	6177.60	1222	3733.60	-883.49	-	-	-
ACE Full (r_A free)	6365.05	1238	3889.05	-841.77	-	-	-
ACE Full (r_C free)	6365.05	1238	3889.05	-841.77	-	-	-
ACE Common	6365.05	1239	3887.05	-845.02	0.00	1	.979
ACE Scalar	6371.95	1241	3889.95	-848.08	6.90	3	.075
ACE Null	6372.02	1242	3888.02	-851.29	6.97	4	.137
AE Scalar	**6372.03**	**1243**	**3886.03**	-854.54	**6.98**	**5**	**.222**
CE Scalar	6380.00	1243	3894.00	-850.55	14.95	5	.011

Table D5: Calculations for standardisation of the mediation model including reaction time variability (RTV), hyperactivity-impulsivity (HI) and emotional lability (EL)

	%	Calculation
Direct effects on RTV		
$A_{C(RTV)}$	8.9%	$A_{C(RTV)}^2/V_{(RTV)} = 0.96^2/10.30 = 0.089$
$A_{S(RTV)}$	34.7%	$A_{S(RTV)}^2/V_{(RTV)} = 1.89^2/10.30 = 0.347$
$E_{S(RTV)}$	56.4%	$E_{S(RTV)}^2/V_{(RTV)} = 2.41^2/10.30 = 0.564$
Direct effects on HI		
$A_{C(HI)}$	11.1%	$A_{C(HI)}^2/V_{(HI)} = 0.96^2/8.33 = 0.111$
$A_{S(HI)}$	52.4%	$A_{S(HI)}^2/V_{(HI)} = 2.09^2/8.33 = 0.524$
$E_{S(HI)}$	34.3%	$E_{S(HI)}^2/V_{(HI)} = 1.69^2/8.33 = 0.343$
Indirect effects on HI		
Correlated effects of A_C with RTV	1.5%	$[[a*A_{C(RTV)}*A_{C(HI)}]*2]/V_{(HI)} = [[0.07*0.96*0.96]*2]/8.33 = 0.015$
Mediated effect of $A_{C(RTV)}$	0.3%	$[a^2*A_{C(RTV)}^2]/V_{(HI)} = [0.07^2*0.96^2]/8.33 = 0.005$
Mediated effect of $A_{S(RTV)}$	0.2%	$[a^2*A_{S(RTV)}^2]/V_{(HI)} = [0.07^2*1.89^2]/8.33 = 0.002$
Mediated effect of $E_{S(RTV)}$	0.3%	$[a^2*E_{S(RTV)}^2]/V_{(HI)} = [0.07^2*2.41^2]/8.33 = 0.003$
Direct effects on EL		
$A_{C(EL)}$	10.3%	$A_{C(EL)}^2/V_{(EL)} = 0.96^2/8.92 = 0.103$
$A_{S(EL)}$	24.9%	$A_{S(EL)}^2/V_{(EL)} = 1.49^2/8.92 = 0.249$
$E_{S(EL)}$	42.2%	$E_{S(EL)}^2/V_{(EL)} = 1.94^2/8.92 = 0.422$
Indirect effects on EL		
Correlated effects of A_C with HI	8.1%	$[[b*A_{C(HI)}*A_{C(EL)}]*2]/V_{(EL)} = [[0.39*0.96*0.96]*2]/8.92 = 0.081$
Correlated effects of A_C with RTV	0.6%	$[[b*a*A_{C(RTV)}*A_{C(EL)}]*2]/V_{(EL)} = [[0.39*0.07*0.96*0.96]*2]/8.92 = 0.006$
Mediated effect of $A_{C(HI)}$	1.6%	$[b^2*A_{C(HI)}^2]/V_{(EL)} = [0.39^2*0.96^2]/8.92 = 0.016$
Mediated effect of $A_{S(HI)}$	7.4%	$[b^2*A_{S(HI)}^2]/V_{(EL)} = [0.39^2*2.09^2]/8.92 = 0.074$
Mediated effect of $E_{S(HI)}$	4.9%	$[b^2*E_{S(HI)}^2]/V_{(EL)} = [0.39^2*1.69^2]/8.92 = 0.049$
Mediated effect of $A_{C(RTV)}$	0%	$[b^2*a^2*A_{C(RTV)}^2]/V_{(EL)} = [0.39^2*0.07^2*0.96^2]/8.92 = 0.000$
Mediated effect of $A_{S(RTV)}$	0%	$[b^2*a^2*A_{(S)RTV}^2]/V_{(EL)} = [0.39^2*0.07^2*1.89^2]/8.92 = 0.000$
Mediated effect of $E_{S(RTV)}$	0%	$[b^2*a^2*A_{S(RTV)}^2]/V_{(EL)} = [0.39^2*0.07^2*2.41^2]/8.92 = 0.000$

Note: % denotes percentage of variance explained in each phenotype; calculations based on statistics in Table 6.6, chapter 6; standardised parameter estimates depicted in Figure 6.5, chapter 6.

Table D6: Calculations for the standardised mediation model including reaction time variability (RTV), inattention (IA) and emotional lability (EL)

Model component	%	Calculation
Direct effects on RTV		
$A_{C(RTV)}$	11.0%	$A_{C(RTV)}^2/V_{(RTV)} = 1.07^2/10.37 = 0.110$
$A_{S(RTV)}$	33.0%	$A_{S(RTV)}^2/V_{(RTV)} = 1.85^2/10.37 = 0.330$
$E_{S(RTV)}$	56.0%	$E_{S(RTV)}^2/V_{(RTV)} = 2.41^2/10.37 = 0.560$
Direct effects on IA		
$A_{C(IA)}$	14.9%	$A_{C(IA)}^2/V_{(IA)} = 1.07^2/7.67 = 0.149$
$A_{S(IA)}$	27.4%	$A_{S(IA)}^2/V_{(IA)} = 1.45^2/7.67 = 0.274$
$E_{S(IA)}$	52.2%	$E_{S(IA)}^2/V_{(IA)} = 2.00^2/7.67 = 0.522$
Indirect effects on IA		
Correlated effects of A_C with RTV	3.6%	$[[a*A_{C(RTV)}*A_{C(IA)}]*2]/V_{(IA)} = [[0.12*1.07*1.07]*2]/7.67 = 0.036$
Mediated effect of $A_{C(RTV)}$	0.2%	$[a^2*A_{C(RTV)}^2]/V_{(IA)} = [0.12^2*1.07^2]/7.67 = 0.002$
Mediated effect of $A_{S(RTV)}$	0.6%	$[a^2*A_{S(RTV)}^2]/V_{(IA)} = [0.12^2*1.85^2]/7.67 = 0.006$
Mediated effect of $E_{S(RTV)}$	1.1%	$[a^2*E_{S(RTV)}^2]/V_{(IA)} = [0.12^2*2.41^2]/7.67 = 0.011$
Direct effects on EL		
$A_{C(EL)}$	13.0%	$A_{C(EL)}^2/V_{(EL)} = 1.07^2/8.84 = 0.130$
$A_{S(EL)}$	37.9%	$A_{S(EL)}^2/V_{(EL)} = 1.83^2/8.84 = 0.379$
$E_{S(EL)}$	42.1%	$E_{S(EL)}^2/V_{(EL)} = 1.93^2/8.84 = 0.421$
Indirect effects on EL		
Correlated effects of A_C with IA	4.4%	$[[b*A_{C(IA)}*A_{C(EL)}]*2]/V_{(EL)} = [[0.17*1.07*1.07]*2]/8.84 = 0.044$
Correlated effects of A_C with RTV	0.5%	$[[b*a*A_{C(RTV)}*A_{C(EL)}]*2]/V_{(EL)} = [[0.17*0.12*1.07*1.07]*2]/8.84 = 0.005$
Mediated effect of $A_{C(HI)}$	0.4%	$[b^2*A_{C(HI)}^2]/V_{(EL)} = [0.17^2*1.07^2]/8.84 = 0.004$
Mediated effect of $A_{S(HI)}$	0.7%	$[b^2*A_{S(HI)}^2]/V_{(EL)} = [0.17^2*1.45^2]/8.84 = 0.007$
Mediated effect of $E_{S(HI)}$	1.3%	$[b^2*E_{S(HI)}^2]/V_{(EL)} = [0.17^2*2.00^2]/8.84 = 0.013$
Mediated effect of $A_{C(RTV)}$	0%	$[b^2*a^2*A_{C(RTV)}^2]/V_{(EL)} = [0.17^2*0.12^2*1.07^2]/8.84 = 0.000$
Mediated effect of $A_{S(RTV)}$	0%	$[b^2*a^2*A_{S(RTV)}^2]/V_{(EL)} = [0.17^2*0.12^2*1.85^2]/8.84 = 0.000$
Mediated effect of $E_{S(RTV)}$	0%	$[b^2*a^2*E_{S(RTV)}^2]/V_{(EL)} = [0.17^2*0.12^2*2.41^2]/8.84 = 0.000$

Note: % denotes percentage of variance explained in each phenotype; calculations based on statistics in Table 6.7, chapter 6; standardised parameter estimates depicted in Figure 6.6, chapter 6.

APPENDIX E

Tables E1-9 present the results of regressions using the covariates age, sex and the eight principal components (PCs) to predict the different thresholds of profile score (thresholds: $p = 1.00$, $p < 0.80$, $p < 0.50$, $p < 0.10$, $p < 0.05$, $p < 0.01$, $p < 0.001$, $p < 0.0001$, $p < 0.00001$). Profile scores were generated in the population target set (TEDS/ SAIL) using the discovery set that included Chinese data.

Tables E10-12 present the results of regressions using the covariates age, sex and the eight principal components (PCs) to predict total ADHD, hyperactive-impulsive and inattentive symptom ratings made using the Conners Parent Rating Scale - Revised (CPRS-R) in the population target set (TEDS).

Tables E13-16 present the results of regressions using the covariates age, sex and the eight principal components (PCs) to predict different informant ratings of ADHD symptoms made using the Strengths and Difficulties Questionnaire (SDQ) hyperactivity scale in the population target set (TEDS).

Tables E17-19 present the results of regressions using the covariates age, sex and the eight principal components (PCs) to predict cognitive performance in a subset of the population target set (SAIL). Cognitive performance measures were reaction time variability (RTV), commission errors (CE) and IQ.

Table E20 presents the results of regressions using the covariates age, sex and the eight principal components (PCs) to predict emotional lability symptoms in a subset of the population target set (SAIL). Emotional lability was assessed using a composite of parent and teacher ratings made using the long version of the Conners Parent Rating Scale - Revised (CPRS-R:L) and the long version of the Conners Teacher Rating Scale - Revised (CTRS-R:L).

All regressions used robust standard errors. The statistics presented are the beta regression coefficient (β), the t test statistic and the two-tailed p value. Two-tailed values were used since there were no directional hypotheses regarding the effects of covariates.

Table E1: Regression of the profile score for the threshold $p = 1.00$ on the TEDS covariates

	β	*t*	*p*
Age	0.002631	0.14	0.886
Sex	0.005204	0.28	0.779
PC 1	-0.019946	-1.13	0.259
PC 2	0.018240	1.05	0.295
PC 3	0.067018	3.75	<0.001
PC 4	0.014025	0.78	0.437
PC 5	-0.103498	-5.66	<0.001
PC 6	0.007490	0.44	0.659
PC 7	0.006673	0.35	0.724
PC 8	-0.029460	-1.57	0.117

Note: The fit of the covariate model was significant, $F(10, 2863) = 5.09$, $p < 0.001$, $R^2 = 0.02$.

Table E2: Regression of the profile score for the threshold $p < 0.80$ on the TEDS covariates

	β	*t*	*p*
Age	0.002330	0.13	0.899
Sex	0.004091	0.22	0.826
PC 1	-0.017759	-1.00	0.315
PC 2	0.020821	1.19	0.232
PC 3	0.066488	3.72	<0.001
PC 4	0.013526	0.75	0.455
PC 5	-0.102334	-5.58	<0.001
PC 6	0.008185	0.48	0.630
PC 7	0.006332	0.34	0.737
PC 8	-0.027465	-1.46	0.144

Note: The fit of the covariate model was significant, $F(10, 2863) = 4.96$, $p < 0.001$, $R^2 = 0.02$.

Table E3: Regression of the profile score for the threshold $p < 0.50$ on the TEDS covariates

	β	*t*	*p*
Age	-0.002008	-0.11	0.914
Sex	0.000977	0.05	0.958
PC 1	-0.013349	-0.75	0.452
PC 2	0.018867	1.08	0.280
PC 3	0.072601	4.10	<0.001
PC 4	0.014204	0.76	0.448
PC 5	-0.096056	-5.22	<0.001
PC 6	0.014943	0.90	0.369
PC 7	0.006055	0.32	0.748
PC 8	-0.021123	-1.12	0.262

Note: The fit of the covariate model was significant, $F(10, 2863) = 4.74$, $p < 0.001$, $R^2 = 0.02$.

Table E4: Regression of the profile score for the threshold $p < 0.10$ on the TEDS covariates

	β	*t*	*p*
Age	0.000123	0.01	0.995
Sex	-0.010429	-0.56	0.576
PC 1	-0.011156	-0.62	0.535
PC 2	0.026063	1.49	0.137
PC 3	0.045475	2.42	0.015
PC 4	0.010249	0.52	0.600
PC 5	-0.078872	-4.39	<0.001
PC 6	0.024023	1.35	0.177
PC 7	0.034070	1.81	0.070
PC 8	-0.005202	-0.28	0.779

Note: The fit of the covariate model was significant, $F(10, 2863) = 3.51$, $p < 0.001$, $R^2 = 0.01$.

Table E5: Regression of the profile score for the threshold $p < 0.05$ on the TEDS covariates

	β	*t*	*p*
Age	0.005998	0.32	0.746
Sex	-0.015366	-0.82	0.411
PC 1	0.008721	0.48	0.628
PC 2	0.012113	0.70	0.485
PC 3	0.041629	2.30	0.021
PC 4	0.000268	0.01	0.988
PC 5	-0.032609	-1.81	0.071
PC 6	0.016030	0.87	0.383
PC 7	0.023795	1.31	0.190
PC 8	-0.001239	-0.07	0.946

Note: The overall fit of the covariate model was non-significant, $F(10, 2863) = 1.31$, $p = 0.218$, $R^2 < 0.01$.

Table E6: Regression of the profile score for the threshold $p < 0.01$ on the TEDS covariates

	β	*T*	*p*
Age	0.024410	1.31	0.189
Sex	-0.012831	-0.69	0.493
PC 1	0.006035	0.33	0.744
PC 2	0.015515	0.83	0.405
PC 3	0.027176	1.50	0.133
PC 4	0.038839	2.00	0.046
PC 5	-0.029566	-1.66	0.097
PC 6	-0.006418	-0.38	0.706
PC 7	0.001714	0.10	0.924
PC 8	-0.030414	-1.65	0.099

Note: The overall fit of the covariate model was non-significant, $F(10, 2863) = 1.49$, $p = 0.136$, $R^2 = 0.01$.

Table E7: Regression of the profile score for the threshold $p < 0.001$ on the TEDS covariates

	β	*t*	*p*
Age	-0.002419	-0.13	0.899
Sex	-0.020106	-1.08	0.281
PC 1	0.054750	2.94	0.003
PC 2	0.019580	1.02	0.309
PC 3	-0.008268	-0.41	0.680
PC 4	-0.014246	-0.73	0.466
PC 5	0.030893	1.65	0.099
PC 6	-0.032745	-1.86	0.063
PC 7	0.008913	0.48	0.631
PC 8	-0.012256	-0.66	0.512

Note: The overall fit of the covariate model was non-significant, $F(10, 2863) = 1.83$ $p = 0.051$, $R^2 = 0.01$.

Table E8: Regression of the profile score for the threshold $p < 0.0001$ on the TEDS covariates

	β	*t*	*p*
Age	0.014298	0.77	0.441
Sex	0.027396	1.47	0.141
PC 1	0.084351	4.64	<0.001
PC 2	0.039314	2.28	0.023
PC 3	-0.045188	-2.56	0.011
PC 4	-0.021886	-1.13	0.259
PC 5	0.048552	2.70	0.007
PC 6	0.002146	0.12	0.904
PC 7	-0.014697	-0.79	0.431
PC 8	-0.000191	-0.01	0.992

Note: The fit of the covariate model was significant, $F(10, 2863) = 4.46$, $p < 0.001$, $R^2 = 0.01$.

Table E9: Regression of the profile score for the threshold $p <0.00001$ on the TEDS covariates

	β	*t*	*p*
Age	-0.0089754	-0.48	0.633
Sex	-0.0071539	-0.38	0.702
PC 1	0.0031524	0.16	0.871
PC 2	-0.0415915	-2.15	0.032
PC 3	-0.0048683	-0.26	0.799
PC 4	-0.0126578	-0.67	0.502
PC 5	0.0602316	3.29	0.001
PC 6	-0.0034356	-0.19	0.850
PC 7	-0.0123821	-0.65	0.514
PC 8	-0.0150730	-0.83	0.406

Note: The overall fit of the covariate model was non-significant, $F(10, 2863) = 1.73$, $p = 0.069$, $R^2 = 0.01$.

Table E10: Regression of CPRS-R total ADHD symptoms on covariates in TEDS

	β	*t*	*p*
Age	-0.113591	-6.24	<0.001
Sex	0.220160	11.74	<0.001
PC 1	-0.015522	-0.82	0.411
PC 2	0.016656	0.89	0.373
PC 3	0.001049	0.06	0.954
PC 4	-0.014775	-0.76	0.449
PC 5	-0.008104	-0.43	0.668
PC 6	-0.001039	-0.06	0.955
PC 7	-0.038342	-2.02	0.043
PC 8	-0.061208	-3.39	0.001

Note: The fit of the covariate model was significant, $F(10, 2682) = 19.40$, $p < 0.001$, $R^2 = 0.07$.

Table E11: Regression of CPRS-R hyperactive-impulsive symptoms on covariates in TEDS

	β	*t*	*p*
Age	-0.123736	-6.53	<0.001
Sex	0.160853	8.49	<0.001
PC 1	-0.014721	-0.79	0.429
PC 2	0.012684	0.70	0.485
PC 3	0.019016	1.07	0.287
PC 4	-0.021894	-1.18	0.239
PC 5	-0.018086	-0.96	0.336
PC 6	-0.010372	-0.53	0.596
PC 7	-0.022815	-1.23	0.221
PC 8	-0.059677	-3.28	0.001

Note: The fit of the covariate model was significant, $F(10, 2681) = 13.11$, $p < 0.001$, $R^2 = 0.05$.

Table E12: Regression of CPRS-R inattentive symptoms on covariates in TEDS

	β	*t*	*p*
Age	-0.083574	-4.61	<0.001
Sex	0.224759	11.97	<0.001
PC 1	-0.008199	-0.43	0.665
PC 2	0.020595	1.09	0.276
PC 3	-0.016107	-0.84	0.400
PC 4	-0.006111	-0.31	0.753
PC 5	-0.001438	-0.07	0.941
PC 6	0.003306	0.18	0.855
PC 7	-0.041503	-2.20	0.028
PC 8	-0.048425	-2.64	0.008

Note: The fit of the covariate model was significant, $F(10, 2684) = 17.90$, $p < 0.001$, $R^2 = 0.06$.

Table E13: Regression of parent-rated SDQ hyperactivity score on covariates in TEDS

	β	*t*	*p*
Age	-0.070096	-3.77	<0.001
Sex	0.231097	12.29	<0.001
PC 1	-0.022177	-1.16	0.246
PC 2	0.011481	0.62	0.537
PC 3	0.013223	0.67	0.500
PC 4	-0.004675	-0.24	0.810
PC 5	-0.017581	-0.89	0.376
PC 6	-0.003857	-0.20	0.841
PC 7	-0.026807	-1.44	0.150
PC 8	-0.029081	-1.63	0.103

Note: The fit of the covariate model was significant, $F(10, 2683) = 17.22$, $p < 0.001$, $R^2 = 0.06$.

Table E14: Regression of teacher-rated SDQ hyperactivity score on covariates in TEDS

	β	*t*	*p*
Age	0.050314	2.42	0.016
Sex	0.263393	12.50	<0.001
PC 1	-0.027943	-1.27	0.203
PC 2	0.006301	0.29	0.770
PC 3	0.020665	1.03	0.305
PC 4	0.000200	0.01	0.992
PC 5	-0.033729	-1.63	0.103
PC 6	0.001515	0.07	0.943
PC 7	0.028578	1.37	0.172
PC 8	-0.020401	-0.97	0.333

Note: The fit of the covariate model was significant, $F(10, 2127) = 17.55$, $p < 0.001$, $R^2 = 0.08$.

Table E15: Regression of child-rated SDQ hyperactivity score on covariates in TEDS

	β	*t*	*p*
Age	-0.023032	-1.19	0.233
Sex	0.173666	9.10	<0.001
PC 1	-0.028348	-1.49	0.137
PC 2	0.014199	0.76	0.445
PC 3	-0.001134	-0.06	0.952
PC 4	-0.003120	-0.17	0.864
PC 5	-0.009244	-0.49	0.626
PC 6	0.038855	1.91	0.056
PC 7	-0.025010	-1.35	0.177
PC 8	-0.045037	-2.44	0.015

Note: The fit of the covariate model was significant, $F(10, 2680) = 9.95$, $p = 0.001$, $R^2 = 0.04$.

Table E16: Regression of multi-rater composite SDQ score on covariates in TEDS

	β	*t*	*p*
Age	-0.007981	-0.36	0.716
Sex	0.293466	13.37	<0.001
PC 1	-0.028477	-1.29	0.199
PC 2	0.018350	0.85	0.397
PC 3	0.022543	1.03	0.305
PC 4	0.000512	0.02	0.981
PC 5	0.002117	0.09	0.926
PC 6	0.034869	1.60	0.111
PC 7	-0.025193	-1.16	0.244
PC 8	-0.042467	-2.04	0.042

Note: The fit of the covariate model was significant, $F(10, 1941) = 18.68$, $p < 0.001$, $R^2 = 0.09$.

Table E17: Regression of reaction time variability on covariates in TEDS (SAIL)

	β	*t*	*p*
Age	-0.310322	-5.15	<0.001
Sex	-0.012762	-0.23	0.816
PC 1	0.007742	0.13	0.894
PC 2	0.119201	2.00	0.046
PC 3	-0.032035	-0.60	0.550
PC 4	-0.025572	-0.51	0.608
PC 5	0.012744	0.24	0.810
PC 6	-0.073026	-1.40	0.164
PC 7	-0.006855	-0.12	0.908
PC 8	0.021865	0.39	0.697

Note: The fit of the covariate model was significant, $F(10, 297) = 3.66$, $p < 0.001$, $R^2 = 0.11$.

Table E18: Regression of commission errors on covariates in TEDS (SAIL)

	β	*t*	*p*
Age	-0.078880	-1.43	0.155
Sex	0.287352	5.30	<0.001
PC 1	0.158812	2.91	0.004
PC 2	-0.003437	-0.07	0.946
PC 3	0.077339	1.39	0.166
PC 4	0.005904	0.14	0.888
PC 5	-0.083622	-1.62	0.107
PC 6	0.058270	1.13	0.261
PC 7	0.013250	0.25	0.807
PC 8	-0.020516	-0.38	0.706

Note: The fit of the covariate model was significant, $F(10, 302) = 4.90$, $p < 0.001$, $R^2 = 0.13$.

Table E19: Regression of IQ on covariates in TEDS (SAIL)

	β	*t*	*p*
Age	-0.190665	-3.45	0.001
Sex	0.092996	1.62	0.107
PC 1	0.005579	0.10	0.917
PC 2	0.041672	0.75	0.454
PC 3	-0.002831	-0.05	0.962
PC 4	0.060244	0.98	0.329
PC 5	0.017916	0.34	0.738
PC 6	0.000677	0.01	0.990
PC 7	-0.026681	-0.52	0.606
PC 8	0.056562	1.05	0.293

Note: The overall fit of the covariate model was non-significant, $F(10, 306) = 1.70$, $p = 0.080$, $R^2 = 0.05$.

Table E20: Regression of emotional lability on covariates in TEDS (SAIL)

	β	*t*	*p*
Age	0.067509	1.05	0.293
Sex	-0.010070	-0.17	0.868
PC 1	0.031661	0.50	0.617
PC 2	-0.088429	-1.54	0.126
PC 3	0.039979	0.69	0.493
PC 4	-0.027493	-0.48	0.633
PC 5	-0.020844	-0.33	0.742
PC 6	-0.135436	-2.03	0.044
PC 7	-0.001692	-0.03	0.976
PC 8	-0.057380	-0.99	0.325

Note: The fit of the covariate model was significant, $F(10, 270) = 1.32$, $p < 0.05$, $R^2 = 0.03$.